Development of a Continuous Flow Sterilisation System Using Microwaves

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: ____March 2017___

ABSTRACT

The focus of this project is to design a continuous flow system to be used for the sterilisation of biological growth media by using only microwaves. The output power of the magnetron has to be controlled in order to control the temperatures to which the media will be heated.

A study was done on different methods of microwave power control and it was found that anode current control is the most suited method. The developed method controls the anode current of the magnetron while keeping the cathode at its constant voltage. Two high voltage transformers used in domestic microwave ovens were connected to the same magnetron. This allowed the anode transformer's supply voltage to be changed by using a triac control circuit. This changes the anode voltage, and so the current, while keeping the cathode constantly heated. Methods of relating the microwave power to a safely measurable voltage or current were also investigated. In the final design, a Hall effect current sensor was used to measure the current on the primary side of the anode transformer and this current was related to the output power of the magnetron.

Temperature sensors to be used for the inlet and exit fluid temperatures were also investigated and PT100 resistance temperature probes were used in the final design. A small peristaltic pump was used to pump the fluid through a PTFE coil inside the microwave cavity. PTFE was selected because it is not susceptible to microwaves and it is chemically non-reactive.

A study was done on the relationship between microwave power and the maximum temperatures reached by the fluid for different flow rates. This was used to develop a control system which was implemented using Matlab and two Arduinos as microprocessors.

The final system was moved to the Biochemistry Department to commence sterilisation tests. Because there are different types of microorganisms, it is important to test for different types as they may react differently to external stimuli.

For this study, both gram negative and gram positive bacteria were tested as well as yeast. The specific gram positive bacteria used was *Micrococcus Luteus*, strain: NCTC 8340; for gram negative bacteria *Escherichia Coli*, strain: DH5α containing a pGKCherry plasmid, and for yeast *Saccharomyces Cerevisiae* was used.

All tests were done in duplicate to confirm the results. The target exit temperature was 90 °C and the flow rate was 3.5 l/h. An initial test was done on *M. Luteus* with a concentration of 10^3 cells per ml. This batch was completely sterile and it was decided to increase the concentration to 10^6 cells per ml for all of the microorganisms tested. All three of these tests achieved sterility.

It was then decided to reduce the temperature to see if this had an effect on the results. These tests were done with *M. Luteus* at a concentration of 10^6 cells per ml. The temperatures selected were 70 °C, 50 °C and 37 °C. Only the 70 °C batch achieved sterility.

It is concluded from this project that continuous flow microwave sterilisation is possible and very effective.

OPSOMMING

Hierdie projek fokus op die ontwikkeling van ʿn kontinue vloei sisteem wat mikrogolwe gebruik om biologiese groei media te steriliseer. Die temperatuur van die vloeistof moet beheer word en dus moet die uittree drywing van die magnetron beheer word. Verskillende drywingsbeheer metodes was ondersoek en daar was gevind dat die beste metode sal wees om die magnetron se anode stroom te beheer terwyl die katode spanning konstant gehou word. Hierdie beheer is geïmplementeer deur twee huishoudelike mikrogolfoond hoë-spannings transformators aan dieselfde magnetron te koppel. Die anode transformator se intree spanning word deur ʿn triac baan beheer wat so die anode spanning, en dus die anode stroom, beheer.

Daar was ondersoek ingestel op verskillende maniere om die drywing te vergelyk met ʿn veilige meetbare spanning of stroom. Die finale ontwerp gebruik ʻn stroommeter om die intree stroom van die anode transformator te meet. Die verband tussen hierdie stroom en die uittree drywing is gedokumenteer.

PT100 weerstand temperatuur probes word in die finale sisteem gebruik om die intree en uittree temperature te meet. Die vloei deur die PTFE spoel binne die mikrogolfoond word gehandhaaf deur ʿn klein peristaltiese pomp. PTFE word gebruik omdat dit nie geaffekteer word deur mikrogolwe nie en dit is chemies onkreatief.

Die verhouding tussen die maksimum bereikbare temperatuur en verskillende drywingsvlakke was gedokumenteer en gebruik in die ontwikkeling van die beheerstelsel. Die beheerstelsel word geïmplimenteer deur gebruik te maak van twee Arduinos en ʿn Matlab program.

Die finale sisteem was geskuif na die Biochemie departement waar die finale sterilisasie toetse gedoen was. Daar bestaan verskillende tipes mikroörganisme en dit is dus belangrik om verskillende tipes te toets omdat hulle drasties anders kan reageer in prosesse.

In hierdie studie was beide gram positiewe en gram negatiewe bakterieë getoets sowel as gis. Die spesifieke gram positiewe bakteria was *Micrococcus Luteus*, stam: NCTC 8340. Vir gram negatiewe bakteria was *Escherichia Coli*, stam: DH5α wat ʻn pGKCherry plasmied bevat gebruik. Laastens vir gis was *Saccharomyces Cerevisiae* gebruik.

Alle toetse was by 90 °C uittree temperatuur en ʿn vloei-tempo van 3.5 l/h getoets. Al die toetse was ook in duplikaat gedoen om die resultate te bevestig. Die eerste toets was *M. Luteus* teen ʿn konsentrasie van 10^3 selle per ml. Steriliteit was bereik in hierdie toets en daar was besluit om die konsentrasie op te stoot na 10^6 selle per ml vir al drie tipes mikroörganismes. Steriliteit was in al drie gevalle bereik.

Die invloed van temperatuur op die sterilisasie proses was ondersoek deur ʿn konsentrasie van 10^6 selle per ml van *M. Luteus* teen die volgende uittree temperature te toets: 70 °C, 50 °C en 37 °C. Die resultate het gewys dat slegs die 70 °C toets steriliteit behaal het.

Die gevolgtrekking van die toetse is dat ʿn kontinue vloei sisteem was slegs mikrogolwe gebruik suksesvol is as ʿn sterilisasie proses.

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Chapter 1:

Introduction

1.1 Background

Microwaves are electromagnetic waves in the frequency range between 300 MHz and 300 GHz and are commonly used in heating processes due to the ability to rapidly increase temperature [1]. Microwave were first invented in 1921 and commercialised around 1940 and are most commonly known for their use in household microwave ovens [2]. However, this is just one of their applications as they are used in many industries including communication, food processing and pharmaceutical production.

In studies and processes relating to microorganisms it is important to be able to grow only a specific type of microorganism. For this, nutrient rich broth is used which is called growth media. The growth media has to be sterile to ensure there is no contaminations in the outcomes of tests or products being produced. Current sterilisation methods only allow batch sterilisation of the media.

A new antibiotic is being developed by the BIOPEP Peptide Research Group lead by Prof. Marina Rautenbach from the Department of Biochemistry, Stellenbosch University. As part of the production line for this new antibiotic, a continuous flow sterilisation system is needed for the growth media used. This project focuses on the development of a continuous flow sterilisation system using only microwaves as a possible solution.

1.2 Objectives

The objectives of this project are:

1. Development of a continuous flow sterilisation system.
   A system should be developed that will sterilise continuous flowing growth media using only microwaves as a source of heat. The microwave output power is to be controlled to ensure that the exit temperature of the media is kept constant.
2. Use of domestic microwave oven components.
   The final system should consist of a domestic microwave oven that is modified to allow the output power to be controlled and continuous flow of the growth media through the microwave cavity.
3. Confirm sterility.
   Sterilisation tests should be done on contaminated media in order to prove that the developed process is a valid method of sterilisation.

1.3 Project Specifications

The growth media has to be kept at a constant high temperature to achieve sterility. Therefore, accurate temperature measurements are an important component of the system and it is desired to have temperature measurements accurate to within 1 °C.

The temperature of the fluid is controlled by the output power of the magnetron. This power should be accurately controlled within 10 W to achieve the correct fluid exit temperature.

Based on the desired output volume of antibiotics, the estimated maximum flow rate of the production line is 5 l/h.

Sterilisation methods are usually assigned a Sterility Assurance Level (SAL) that gives an indication as to how well the method works. Generally a SAL of 10^{-3} is the minimum level that is accepted for sterilisation but for pharmaceutical applications a SAL of 10^{-6} is preferred.

1.4 Project Development

The development process of the project is shown in Figure 1.1.

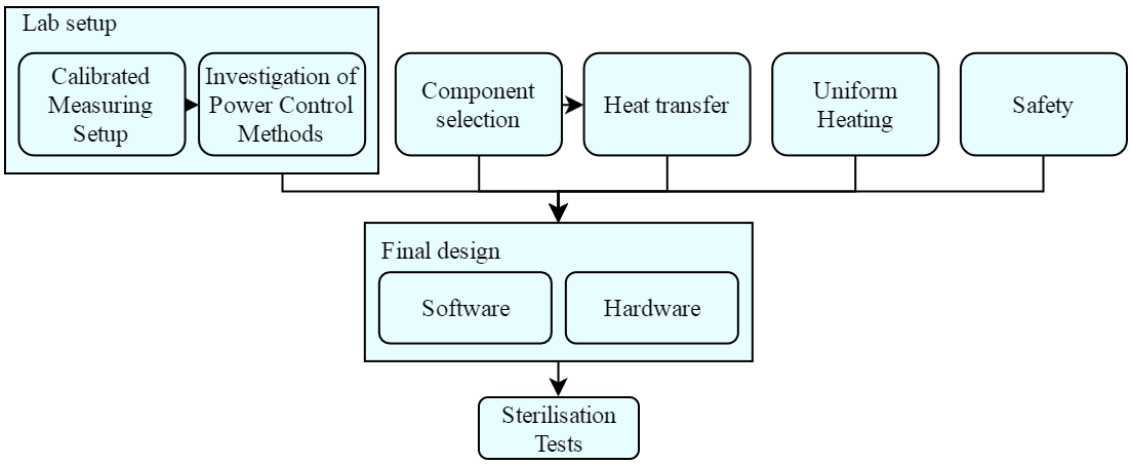


Figure 1.1: Project flow

Initially a measurement setup is constructed using calibrated equipment to measure the output power of a magnetron accurately. This calibrated measurement setup is used to investigate magnetron output power control methods. These methods are developed using components from a domestic microwave oven.

Methods of temperature measurements are investigated to achieve accurate temperature readings within the difficult measuring environment surrounding a microwave oven. These measurement methods are used to confirm the heat transfer rates that are calculated after the final coiled pipe is designed.

A reason for non-uniform heating within a microwave oven is discussed and a method of improving uniform heating is implemented. The safety concerns of this type of project are documented and precautions to be taken are given.

Knowledge gained from these different sections will be used in the development of the final system. The software of the final system includes a designed PI-controller for the output microwave power as well as the software for all the different sensor measurements required. The final hardware is a unit that includes a modified domestic microwave oven, a pump, sensors and the circuits required.

Finally, tests will be done on contaminated growth media to prove that sterility is achieved using the developed microwave system.

1.5 Thesis Format

Chapter 2: The literature study gives an overview of microwaves, how they are generated and how microwave power is controlled in industry. This chapter also discusses microwave sterilisation currently used in industry. Descriptions of the currently used sterilisation methods in microbiology are also discussed.

Chapter 3: This chapter describes the calibrated measurement setup that is used to develop the output power control method. This developed method is discussed in detail and methods of relating power to voltage or current are investigated.

Chapter 4: The coil used in the modified microwave setup is designed in this chapter. The selection and calibration of temperature sensors is also described. A pump for the final system is selected and the initial domestic microwave setup is assembled.

Chapter 5: The non-uniform heating effect cause by standing waves is discussed. Methods used to visualise these waves and how to improve uniform heating is described.

Chapter 6: The heat transfer in the microwave system is calculated which is used in the design of the control system. Heat transfer of a similar system that uses steam instead of microwaves to achieve the final temperatures is also calculated. This is done to compare the maximum temperatures achievable by both systems.

Chapter 7: In this chapter a PI control system is designed for the final continuous flow system. This chapter also discusses the adjustments made to compensate for the initial start-up of system where the fluid exit temperature is unknown.

Chapter 8: The final system layout is discussed along with the circuits used. The software that controls the system is also explained and sample test results of the system is shown.

Chapter 9: The preparation for the sterilisation tests as well as the test procedures are discussed. The results of these tests are given and the developed method of sterilisation is compared to the currently used method.

Chapter 10: Recommendations for future development of this concept are given and the project is concluded.

Appendix A: General safety procedures are discussed that can be applied to similar projects in future.

Chapter 2:

Literature Study

2.1 Chapter Summary

This chapter discusses what microwaves are and how the output power of a magnetron of a microwave oven can be controlled. Factors influencing microwave heating are discussed along with its application in industry. Sterilisation is defined and microwave sterilisation methods used in the food processing industry are discussed. Problems identified with the use of microwaves in sterilisation processes are also given. In addition, a detailed discussion of sterilisation methods currently used in the microbiology field is provided.

2.2 Microwaves

This section discusses what microwaves are and how they are generated. Magnetron output power control methods are also discussed and are used in Chapter 3: Measurement Setup and Microwave Power Control.

Heating applications have been limited to a few frequency bands for industrial, scientific and medical use, known as the ISM band. This was done to prevent interference with radio frequencies used in telecommunication. The frequency bands commonly used for microwave heating are 915 MHz and 2.45 GHz, and according to international convention, microwave ovens used for domestic applications operate at 2.45 GHz [1].

A microwave oven consists of three main components: a high voltage power supply, a magnetron and the cavity containing the product to be heated. Microwaves are generated

by the magnetron and are directed into the cavity. The cavity is made of metal walls, usually aluminium, which act as a Faraday cage, keeping the waves inside [1].

2.2.1 Magnetron Operation

The magnetron is the source of the microwaves and operates at its designed frequency. An illustration of the construction of a cavity magnetron can be seen in Figure 2.1.

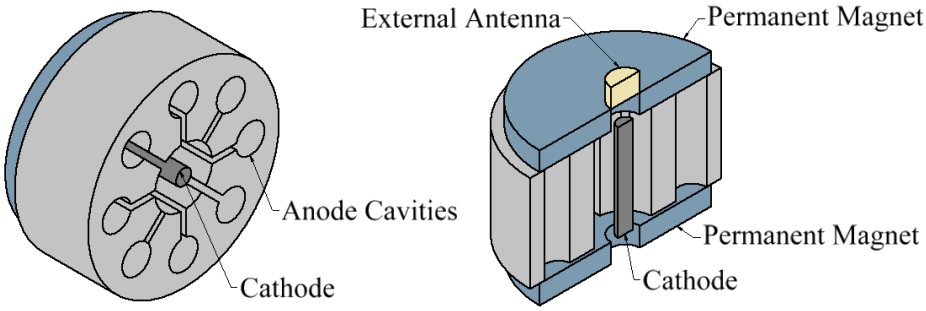


Figure 2.1: Magnetron design [11]

A magnetron consists of a cathode (filament), an even number of anode cavities, two permanent ring magnets and an external output antenna [2]. Microwaves are created by electrons that are emitted from the cathode and follow a path to the anode [3]. The anode cavities are designed to resonate at a specific frequency, for example 2.45 GHz.

A magnetic field is created parallel to the central axis by placing permanent magnets across the chamber. An anode voltage of several kilovolts is applied to create an electric field that is perpendicular to the magnetic field [1]. A low voltage and high current is applied to the cathode to heat it up which then releases the electrons. Instead of moving in radial lines the electrons start to move in circular patterns due to the applied magnetic fields. These electrons then start to oscillate at the designed frequency within the anode cavities [3]. Microwave power is extracted by small coupling antennas in the cavities which are then connected to the external antenna which transmits microwaves into the oven cavity or waveguide [1].

Since a magnetron has the same VI characteristics of a diode they are classified as groups of diode-type electron tubes. This relationship is piecewise linear and is divided into two areas: non-oscillating and oscillating as shown in Figure 2.2.

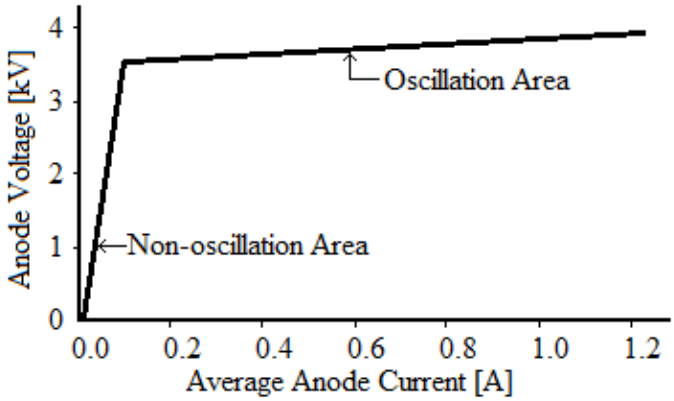


Figure 2.2: VI characteristics of a magnetron [3]

The anode voltage determines which area the magnetron is in [3]. The switch between the two areas occurs at a very specific voltage, *Vz*, which is usually between 3-4 kV. This voltage differs depending on the magnetron design and construction. The equivalent circuit of a magnetron has two branches to represent the two operating regions as seen in Figure 2.3.

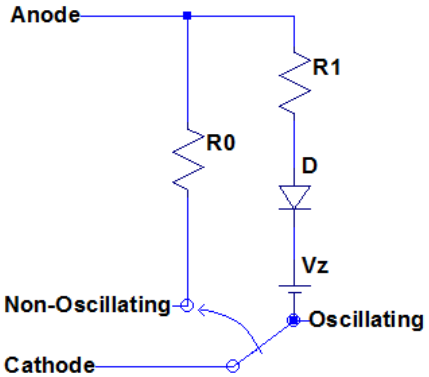


Figure 2.3: Equivalent circuit of a magnetron [3]

For the oscillating area the resistance is low and the diode is forward biased, meaning the magnetron is switched on. In the non-oscillating area the resistance is high and the diode

is reversed biased, so the magnetron is off [3]. This on-off cycle of the magnetron is at the supply voltage frequency, which for this project is 50 Hz.

2.2.2 Magnetron Thermal Drift

Magnetron thermal drift is when the power output of a magnetron changes due to the temperature of the magnetron. This occurs because the anode of the magnetron gets bombarded with electrons at the anode tips which causes it to heat up. This changes the thermal profile of the entire anode-cathode structure. The output frequency of the magnetron drifts as the components reach their operating temperature, which causes the output power to change. The maximum drift occurs in the first few minutes after the magnetron is switched on. Equilibrium is reached after 10-30 minutes, depending on the magnetron design. Subtle differences in the design or construction of the magnetron can lead to extreme differences in thermal drift curves. It is worth noting that thermal drift occurs whenever the anode current is changed and not just at start-up. Magnetrons also have temperature coefficients that indicate thermal drift after thermal equilibrium has been reached. This is caused by changes in the ambient temperature that change the temperature of the magnetron [4].

2.2.3 Magnetron Power Control

The output power of a magnetron is proportional to the average anode current [5]. This anode current dominates the power of the magneton and must be finely controlled as irregular currents will shorten the magnetron life [2].

There are three control methods that are commonly used to control the magnetron output power. The most commonly used method of microwave power control is the use of duty cycles [3]. This method is applied in most domestic microwave ovens and produces microwave pulses. With this method, the magnetron is switched on for a period and then switched off for a period.

An example of this method's output power is shown in Figure 2.4 where the magnetron is on for 16 seconds then off for 4 in a repeating 20 second cycle. This leads to an average output power of 80% of the maximum power.

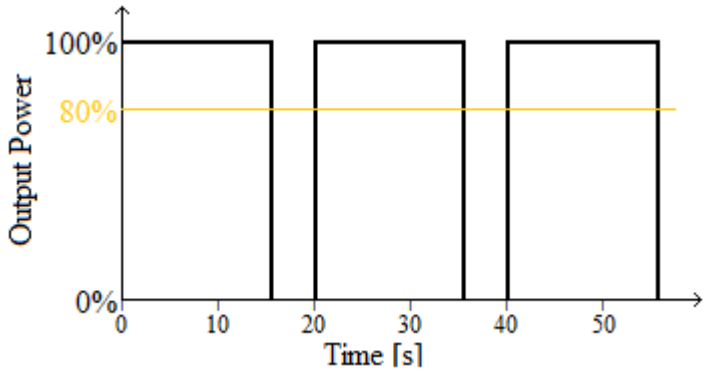


Figure 2.4: Duty cycle control yielding 80% average output power

This method results in lower average output power over time, but this is not continuous power. This method is not applicable for this project since the power during the on cycle will be too high and the off cycle will leave growth media unsterilised.

The second microwave power control method is the use of electromagnets to control the magnetic field across the anode [5]. This method can be cost-effective and provides continuous output power. This is mostly used for high power magnetrons as efficiency is greatly reduced at lower power levels.

Lastly, the anode current can be controlled by controlling the anode voltage which will produce continuous output power. This method however may not be suitable for microorganism sterilisation where duty cycle controlled microwaves are more effective due to the higher death rates that can be achieved. Studies have found a 40% increase in death rate when pulsed microwaves were used instead of continuous microwaves [5]. This may be compensated for by increasing the exposure time.

There have been many studies using anode current control implemented in different ways. These methods can be implemented on either the low or high voltage side of the transformer used to power the magnetron. Figure 2.5 shows the general magnetron power supply circuit with these areas indicated.

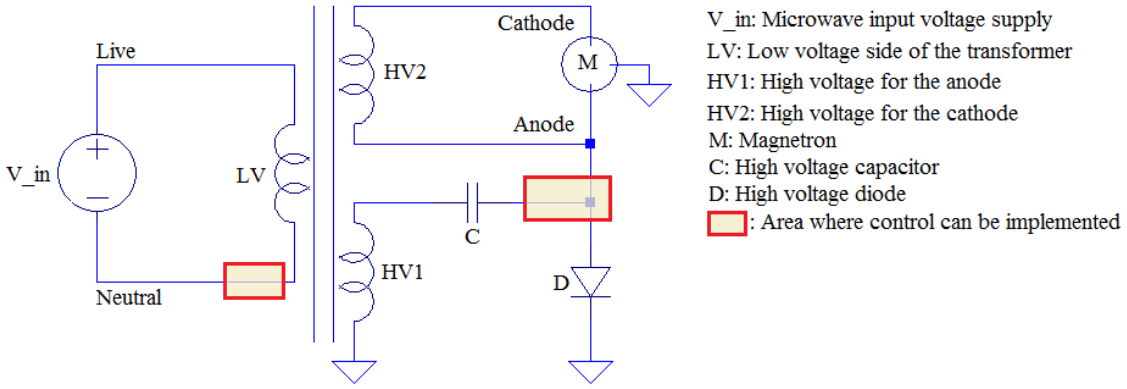


Figure 2.5: Magnetron power supply circuit with anode current control implementation areas

One way of implementing anode current control is an approach that uses a triac controller to control an electronic variator's conduction angle at the low voltage side of the transformer and uses the DC high voltage after the capacitor as a trigger signal. This is used to limit the anode voltage and thus the average output microwave power [5]. Another method used a triac phase controller at the low voltage sides to change the phase of the input voltage to reduce the current to the anode [3]. Other methods have implemented soft switching inverter circuits and switch mode power supplies, but these circuits can be expensive to develop and difficult to control [2] [6]. Many of these methods use a separate transformer for the cathode voltage and all of these methods produce continuous output power.

This project will develop a circuit to implement anode current control which involves the use of an AC voltage controller triac circuit. These types of controller circuits allow for complete linear control of resistive loads, but is non-linear for inductive loads. It has been shown that there is a linear region to which the power control can be applied [3]. The high voltage transformers that will be used are complex loads and may not react to the AC voltage controller linearly.

2.3 Microwave Heating

This section covers the theory that will be used to improve the design of the system for optimal heating which is discussed in Chapter 6: Heat Transfer.

Two methods of absorbent heating are convective heating and microwave heating. Convective heating heats a target from the outside inwards whereas with microwaves, heat can be produced deeper within the object due to the wave's penetration ability. The penetration depth of the microwaves is determined by the frequency as well as the material properties of the target to be heated.

In microwave heating, heat is commonly produced by the oscillation of water molecules. This oscillation also occurs in materials containing hydroxyl groups such as sugars, fats and most forms of plastics [7]. Water or hydroxyl groups consist of polar molecules that try to align or follow the applied oscillating electromagnetic (EM) field. This rapid change, which is at the operating frequency of the magnetron, causes heat to build up due to friction between the molecules.

The effects of this applied EM field are divided into two groups. First are factors that depend on the dielectric properties of the irradiated material in the form of heat - this is called the thermal effect. Secondly are the factors that do not depend on the heat produced, but are rather a direct effect of the EM frequencies applied to the material and this is known as the non-thermal effect of microwaves [8].

There have been many studies and much speculation about the existence of the non-thermal effect, yielding conflicting conclusions. In liquids the temperature rises due to dielectric absorption as this converts electrical energy into heat. The heat produced can mask the non-thermal effects microwaves may have. An attempt to differentiate these effects on microbial compositions in dry materials was made and the study found that there are no non-thermal effects in dry materials [8]. Despite many studies on microbial destruction by microwave radiation the mechanism of destruction is not fully understood, but it is generally accepted that it is mainly due to the thermal effects of microwave exposure [9].

Domestic microwave ovens allow batch heating, but some industrial applications incorporate microwaves as part of a continuous heating process. Microwaves are used in many other fields, including pasteurisation of milk and vegetables, drying of textiles and paper and the thermal treatment of pharmaceutical products [1]. Microwaves have been used to temper frozen ingredients and also for the drying of foods without great success [10]. Microwaves have also been used, with some success, in the treatment of waste water [9]. However, microwave sterilisation has had limited success in the past and has had multiple problems [11].

One of the problems that has been identified when using microwaves for sterilisation includes the non-uniform temperature distribution that is produced in mixed materials due to differing material properties such as moisture content. There is also an unequal temperature distribution as well as variation in thermal efficiency in irregularly shaped materials. If larger products are not properly insulated the temperature rise on the surface of the object may be slowed due to radiation heat loss from the surface. This means the surface of the object may take longer to be sterilised [12]. It is because of these reasons that microwaves are often combined with other heating methods such as UV light, pressurised steam, hot water or even microwave plasma [12] [13] [14].

2.3.1 Factors Affecting Heat Transfer in a Microwave Cavity

EM waves can be absorbed by matter in many ways depending on the state of the matter. Solids and liquids can absorb microwave energy due to the polarisation caused by the external oscillating EM field [1]. Most of the microwave energy is absorbed by the polar molecules present in the target being heated.

There are many factors influencing the absorption of microwaves and thus the heating capability of microwaves. These factors are discussed briefly below.

Frequency: The wavelength determines the penetration depth which determines whether or not the target will be heated throughout [11]. At lower frequencies the dipole orientation can follow the alternating field in phase, but as the frequency increases the inertia and friction between the molecules make it more difficult for the dipoles to follow the field and they start to lag. This phase lag absorbs power from the field and this is

known as dielectric loss caused by dipole relaxation. At very high frequencies, 1 - 10 THz, the molecules no longer respond to the changes in the field [1].

For water, the penetration depth of microwaves at 2.45 GHz is in the order of centimetres, which is ideal for domestic microwave oven since the target's dimensions are usually in that range [1]. Higher penetration depths can be reached in the 915 MHz, 8 - 22 cm, as opposed to the 2.45 GHz band that only has a penetration depth of 3 - 8 cm depending on the moisture content [11].

Dielectric properties: These are the electrical properties of the material in context of microwave and radio frequencies and gives a measure of how well the target will interact with the electromagnetic energy. Biological material has the ability to store and dissipate electrical energy. This can be expressed by the dielectric permittivity, ε, where the real part is the dielectric constant, ε', and the imaginary part is the dielectric loss, ε'' [11].

$$\varepsilon = \varepsilon' + j\varepsilon'' \qquad \textbf{2-1}$$

This complex permittivity is a measurement of the materials capability to couple electrical energy produced by the magnetron. The loss tangent is an indication of the material's susceptibility to be penetrated by an electric field and dissipate energy as heat. PTFE and glass have low loss factors which means they absorb very little microwave energy. These materials are used to make containers that can be used inside the microwave oven [11].

Moisture content: One of the main factors in microwave heating is the water content of the target. This affects the dielectric properties of the material and changes the penetration depths. Higher moisture content targets have smaller penetration depths and this leads to non-uniform heating [11].

Volume: There is a direct relationship between volume and absorption of microwaves. Continuous systems may provide more uniform heating as the product is moved through the microwave field. The critical minimum sample mass for efficient operation for a domestic microwave oven is 250 ml water load per 1 kW output power. Below this level a significant amount of power is unabsorbed and reflects back to

the magnetron. This reflected power can cause damage to the magnetron and reduce its efficiency [11].

Temperature: The sample's temperature affects the microwave heating and may change the dielectric properties. Temperature and moisture content can change during heating and this may have a combined effect on the dielectric properties and thus the heating behaviour [11]. The temperature rise caused by microwaves depends on the initial temperature of the target [1]. The initial temperature should be known so the power can be adjusted accordingly. Higher initial temperatures can be compensated for by reducing the power, using a larger sample volume or reducing the exposure time.

Geometry and location: The shape of the target affects the penetration depth and this will cause non-uniform heating in irregularly shaped targets. Spherical or cylindrical shapes will heat up more evenly as the higher surface to volume ratio increases the heating rate. The relationships between load geometry, orientation and oven cavity parameters has not yet been fully established [11].

Thermal properties: Properties such as thermal conductivity, density and heat capacity affects heating characteristics of the target. Higher thermal conductivity allows faster heat dissipation and lessens the time it takes to reach a uniform temperature. Added solid content like salt or protein can increase heat capacity. Heat capacity measures the temperature response to heat input or removal. The combination of heat capacity, thermal conductivity and density is represented by thermal diffusivity. This is defined as the ratio of thermal conductivity to the target's volumetric heating capacity [11].

Secondary flow in coiled pipes: Thermal processing requires the coldest point in the fluid to reach a target minimum temperature for a specified holding time. The coldest point in flowing fluids is where the fluid reaches its maximum velocity. In a straight pipe this point is located along the central axis, but for a coiled pipe this point varies, and because of this the flow characteristics should be determined. Helical coils create secondary flow due to centrifugal force and this ensures better mixing and stabilises laminar flow [11].

2.4 Sterilisation in Industry

The theory discussed in this section relates to the final sterilisation tests done at the end of the project with the developed system. These tests and their results are discussed in Chapter 9: Sterilisation tests.

2.4.1 Microwave Sterilisation

Pasteurisation is a process that uses mild heat to kill pathogens and inactivate vegetative bacteria and enzymes in food. This process is commonly used on milk and fresh fruit juice to eliminate any potential health hazards. Bacterial spores are not killed by this process and this reduces the product's shelf life at room temperature. Sterilisation is a process that kills nearly all living microorganisms in a substance. Sterilisation is a more severe heat treatment that leads to commercial sterility of products and gives them a longer shelf life. Commonly, saturated steam at high pressure and heated water baths are used to sterilise the products [11].

Heat sterilisation is often used in the food industry and is based on exposing food to high temperatures for a period of time. Since the food is then only heated from the outside, the inside would not be sterilised in shorter treatment times. However, longer treatment times causes the quality of food to deteriorate due to nutrients being destroyed. Instant heat sterilisation methods are required to completely sterilise microorganisms without damaging the product. This is where microwaves may be extremely useful [12].

The first microwave sterilisation system to be approved by the US FDA was developed by Washington State University and was approved in 2009 [10]. This system submerges the packaged food into pressurised hot water and simultaneously radiates the packages with microwaves at a frequency of 915 MHz [13].

It has been found that microwave pasteurisation, keeping milk at 72 °C for 15 sec, reduced the bacteria, psychotropic bacteria counts, E-coli and alkaline phosphates [15]. It has also been reported that microwave pasteurisation is faster than conventional methods for deactivating alkaline phosphates, which is the most heat resistant enzyme in milk, and

reduces the number of foodborne microorganisms [16]. If microwaves can be successfully used for pasteurisation then it should be possible for sterilisation to be achieved as well.

2.4.2 Sterilisation in Microbiology

In the microbiology field growth media is used to grow specific organisms in or to test new medicines such as antibiotics on specific microorganisms. It is therefore important that the media is sterile to ensure no other organisms grow that would invalidate test results. There are three categories of sterilisation methods: chemical, filtration and heat [17].

Chemical sterilisation by gas uses ethylene oxide mixed with carbon dioxide to sterilise equipment or containers. This gas is used at low temperatures of 25 °C – 55 °C and is highly effective in killing microorganisms including heat resistant spores. This method consists of a three stage cycle, which lasts around 14 hours per cycle. Ethylene oxide is also highly flammable and toxic which means this method requires very strict safety procedures [18]. Chemical solvents such as ethanol and chloride are also commonly used to sterilise surfaces or tools and to sterilise used growth media before it can be discarded safely.

Filtration sterilising methods are mostly used to sterilise heat sensitive liquids by passing it through bacterial filters. This process is very effective, but can be slow due to clogging in the filters. Care must be taken to replace filters before they rupture and contaminate the already sterile liquid [17]. Filtration can also be a very expensive process [19].

Heat sterilisation is the most common method used and has two subcategories: dry heat and wet heat. Dry heat methods include flaming and incineration, which is the process of exposing metal or glass tools to a direct flame to burn off all microorganisms [17] as shown in Figure 2.6.

Figure 2.6: Streaking tool being flamed before use

Flaming is also used when working with sterile containers by briefly exposing the opening of the container to a flame to eliminate any potential contaminants on the rim. This is done each time the container is opened or closed.

Dry air ovens are used to sterilise powders, metal tools and glassware. These ovens can be set to a specific temperature and are kept there for a set time by circulating dry hot air [17].

Radiation is considered a form of dry heat sterilisation and kills microorganisms by breaking down the DNA [20]. This can be divided into ionising and non-ionising radiation. For non-ionising radiation, UV light is commonly used. UV is mostly used for sterilising work chambers and surfaces since it has a short penetration depth. It can also be used in doorways to prevent any airborne microorganisms from entering a room. UV is also the safer form of radiation to use [17].

Ionising radiation includes X-rays and Gamma-rays with longer penetration depths which makes it very dangerous to work with. Special rooms and clothing are used for this method of sterilisation, but it is highly effective for large scale sterilisation [20].

Wet heat sterilisation includes boiling items to kill microorganisms or exposing them to steam [17]. Autoclaves are the most common form of wet heat sterilisation. These devices

use pressure to super heat steam to bring the temperature up to approximately 121 °C. The chamber is then kept at this temperature for a set time between from 15 min - 30 min before the pressure is reduced and the chamber starts to cool down. An autoclave cycle can take over an hour to be completed. The autoclave chamber can be filled with metal and most plastic instruments, glassware and liquid media together.

Focusing on the sterilisation of growth media, autoclaving is the most commonly used method due to its good results and relative ease of use. There is an element of danger to them due to the high pressure steam that is used. Operating instructions should be followed carefully and regular maintenance of the seals should be performed.

Autoclaving helps in the hydrolysis of proteins which releases amino acids and improves growth media quality [19]. Autoclaving also has negative effects on the media; due to the high temperature and long exposure time caramelisation can occur which is the oxidation of sugars [21]. This degrades the nutrient levels of the media and causes it to turn darker gold in colour [19]. During heating the Maillard reaction also takes place in the media. This is the reaction between the amino acids released during the hydrolysis of proteins and reducing sugars, which also causes the media to turn darker gold in colour [19]. The breakdown of an amino acid called *tryptophan* is also a concern with longer autoclave times as this is an important nutrient in the media [22].

The severity of each of the above described effects differs with every use of the autoclave [19]. This is because the autoclave cavity can be filled with different tools and containers each time, which means the heat distribution within the media is not always the same. A small container of media will reach the maximum equilibrium temperature faster than a larger container in the same autoclave.

Figure 2.7 shows one of the autoclaves at the Biochemistry Department as it was being unpacked after a cycle.

(a) (b) (c)

Figure 2.7: (a) Autoclave sealed shut during operation; (b) All tools and containers on a trolley inside the chamber; (c) Close-up of a the fully packed trolley with different tools and liquids

The autoclave is very good at killing microorganisms, but to kill fungi spores the steam needs to be in direct contact with the object being sterilised [23]. This raises a problem with media being sterilised in sealed containers and can lead to media that may still contain some active spores [19].

2.5 Chapter Closing

This chapter discussed microwaves and their heating applications in industry. Output power control methods were also discussed. Pasteurisation and sterilisation was differentiated and the problems identified in microwave sterilisation were discussed. The next chapter will lead in the project by discussing the power control method developed for this project.

Chapter 3:

Measurement Setup and Microwave Power Control

3.1 Chapter Summary

This chapter will focus on the calibration of a laboratory measurement setup as well as the development of the microwave power control method. Figure 3.1 shows this chapter as part of the overall development of the final system.

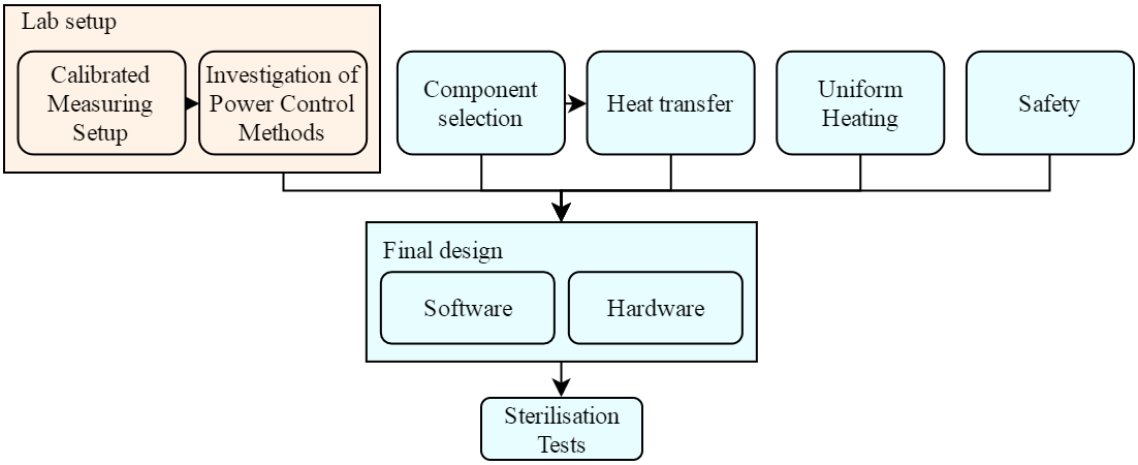


Figure 3.1: Project development block diagram with the current chapter's work indicated

The specific layout of the chapter can be seen in Figure 3.2.

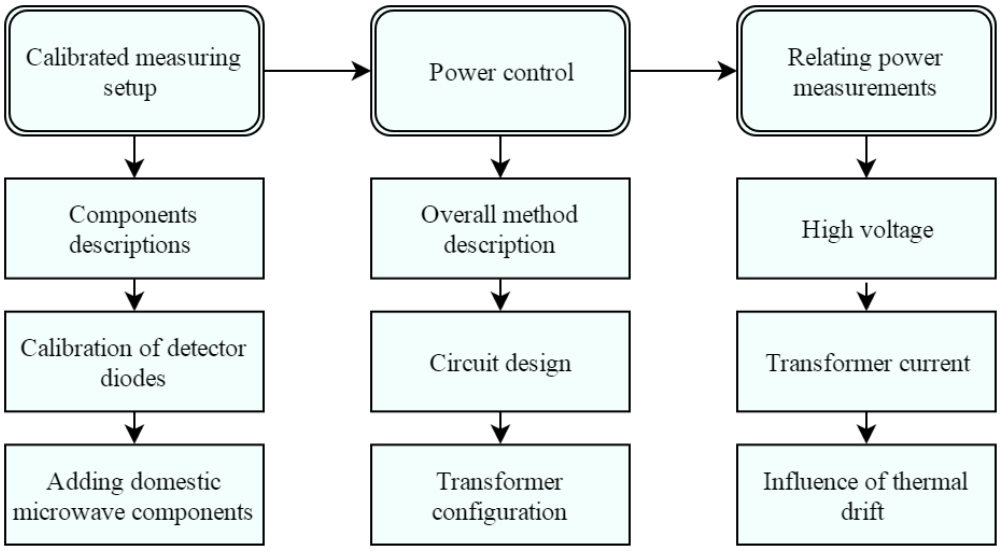


Figure 3.2: Chapter structure

Firstly, the components of the measurement setup are described and the calibration of the detector diodes is discussed. This measurement setup is then used to develop a power control method to implement anode current control on domestic microwave oven components. The circuits used are discussed and the optimal transformer configuration is investigated to determine the best one for the final system.

Methods of relating the magnetron output power to either the anode voltage or transformer current are investigated. Finally, the thermal drift of the magnetron is measured to be incorporated in the final design.

Before any tests could be started a safety analysis was done on the lab and the setup. A general safety analysis and precautions can be found in Appendix A: Safety.

3.2 Calibrated Measurement Setup

The measurement setup was assembled using existing components from the microwave heating research group. These components are calibrated measurement equipment that will be used to accurately measure the microwave power. This setup consists of an industrial magnetron and its fan, a high voltage power supply, an isolator, a dual directional coupler and an applicator connected as seen in Figure 3.3.

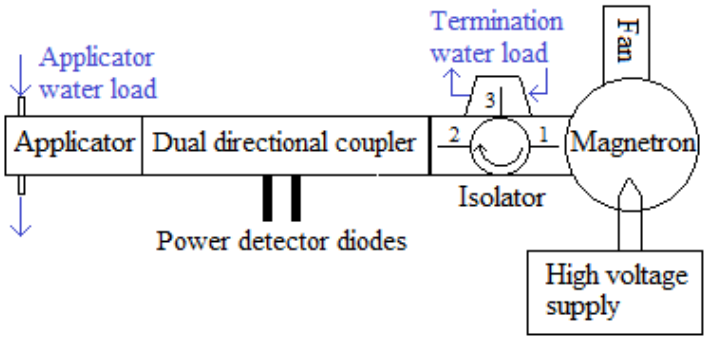


Figure 3.3: Measuring setup

The magnetron generates the microwave power into the system and an isolator is placed in front of the magnetron to absorb any reflected power to protect it. The microwave power is measured by the power detector diodes in the dual directional coupler, also known as a bi-directional coupler. An applicator is a chamber that safely contains the microwaves as well as the material to be heated. The applicator used in this setup is a modified waveguide that allows a small pipe to be passed through the microwave cavity without any microwave leakage. In this setup the power that is not absorbed by the applicator load is reflected back and absorbed by the isolator. The magnetron and its power supply used in this system has 1.3 kW output power. This magnetron is used to confirm the calibration of the detector diodes since its power is known. The physical setup is shown in Figure 3.4.

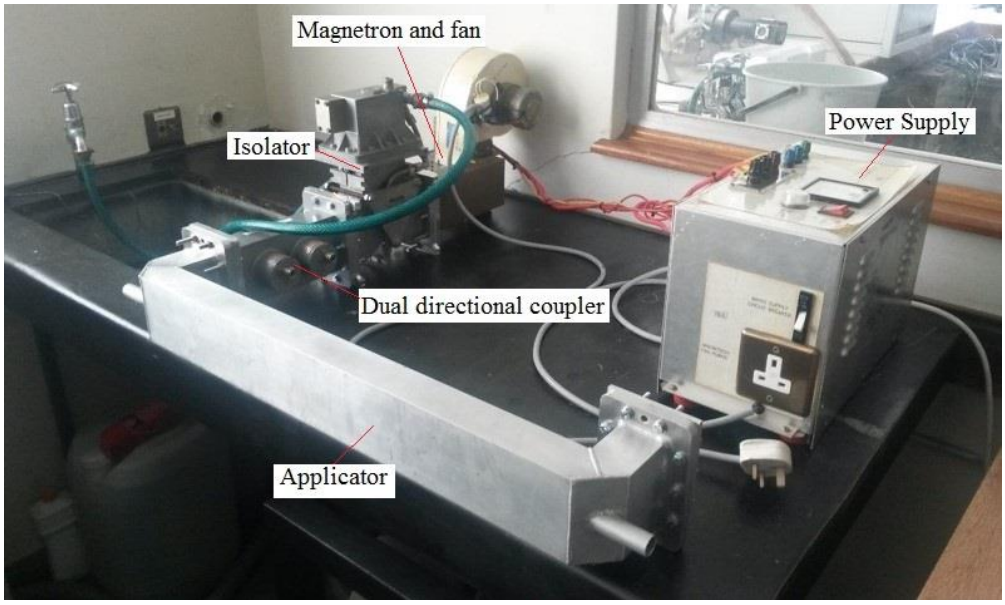


Figure 3.4: Calibrated measuring setup

3.3 Details of Measurement Components

The magnetron is connected to the high voltage power supply and a fan is used to keep the magnetron from overheating. The isolator provides 20 dB isolation to protect the magnetron from reflected waves. The isolator is a three port device that allows microwaves to move from port 1 to port 2 and from port 2 to port 3. However, almost no waves can pass from port 2 back to port 1 as port 3 has water flowing through it as a load to absorb microwaves reflected back from the applicator. A visualisation of an isolator is shown in Figure 3.5.

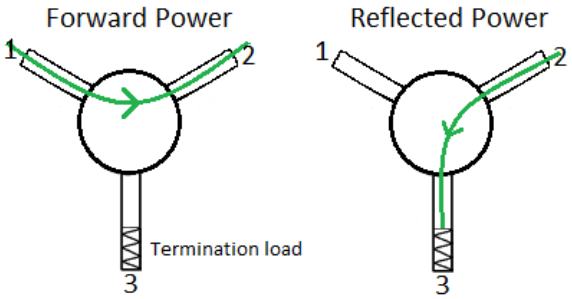


Figure 3.5: Power flow within an isolator

The 60 dB dual-directional coupler can measure forward and reflected power. The two detector diodes converts the measured power into an equivalent voltage which can be measured using an oscilloscope. A close-up photo of the coupler with the connected diodes is shown in Figure 3.6.

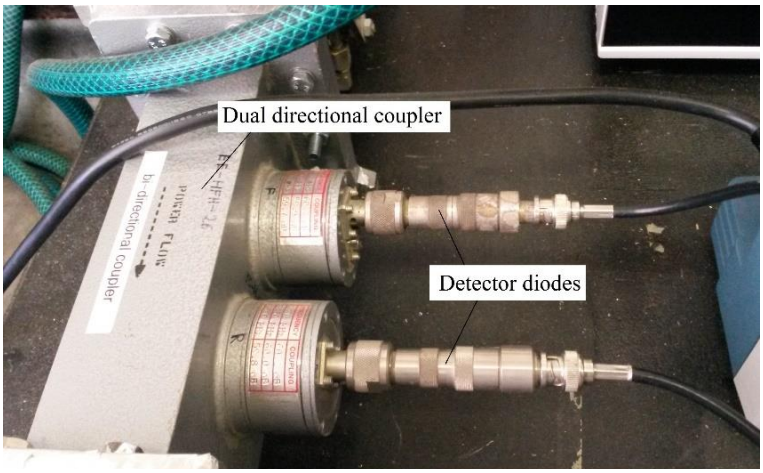


Figure 3.6: Dual directional coupler with power diodes connected

An example of the measurements using the detector diodes is shown in Figure 3.7.

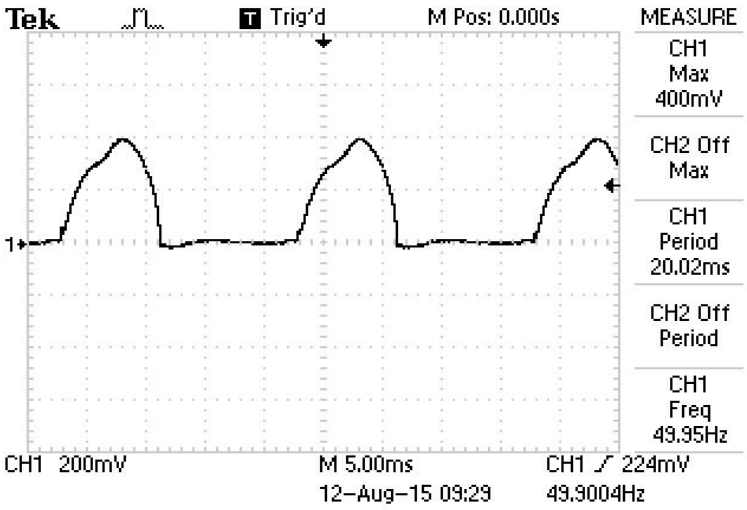


Figure 3.7: Magnetron output power measured with a detector diode

3.3.1 Detector Diode Calibration

The power detector diodes were calibrated outside the measurement setup using a signal generator that allowed adjustable power output. The diodes were connected to the signal generator at 2.45 GHz and the power was increased from -20 dBm to 10 dBm in 1 dBm increments. The output voltage was measured after each power increase as shown in

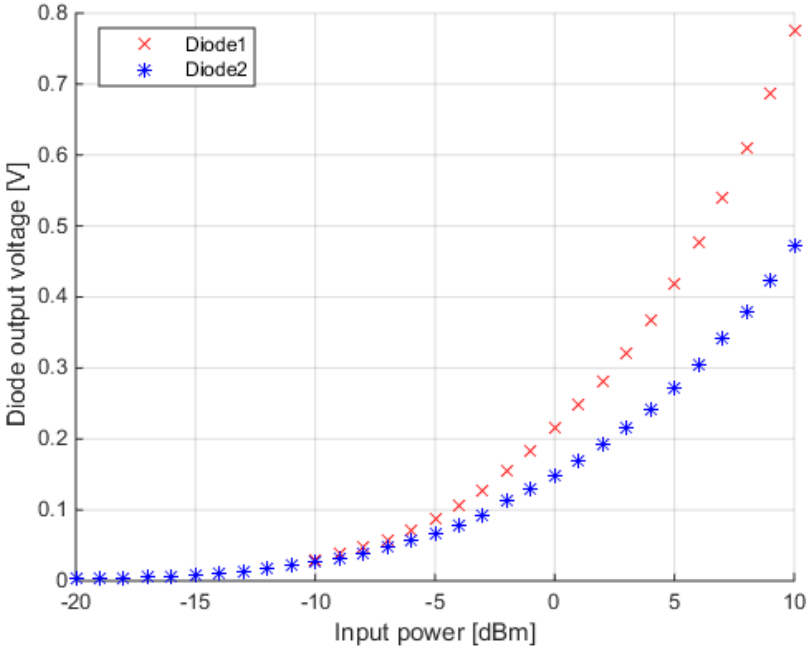


Figure 3.8: Measured diode output voltages

Figure 3.8.

These results were used to determine a calibration curve for each diode by first converting the dBm power to Watts using the following equation:

$$P_{(W)} = \frac{10^{\frac{P_{(dBm)}}{10}}}{1000} \qquad \textbf{3-1}$$

Matlab was used to determine the function that best describes these curves which will be used in future conversion calculations to relate the power diode readings back to measured microwave power. The calibration curves for the diodes can be seen in Figure 3.9 with both the measured and fitted curves.

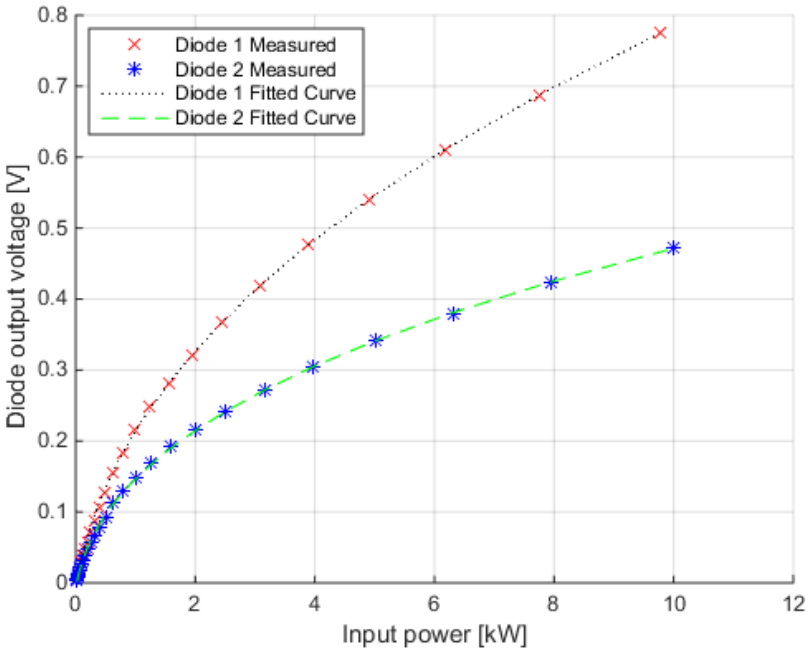


Figure 3.9: Diode calibration curves

3.3.2 Modified Measurement Setup

The calibrated measurement setup was adapted to use domestic microwave oven components to develop the power control method. The industrial magnetron, fan and power supply were replaced with those of a domestic microwave oven as these are the components that will be used in the final system. The modified measuring setup can be seen in Figure 3.10.

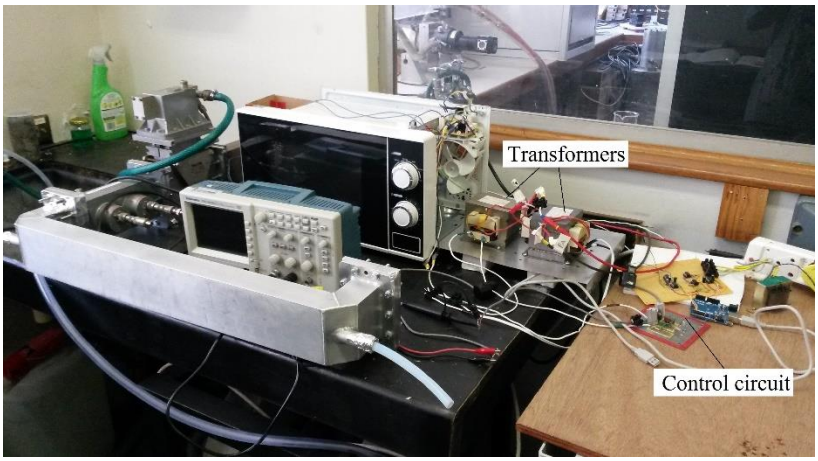


Figure 3.10: Modified measuring setup with domestic microwave oven components

An isolation transformer was necessary for the oscilloscope to perform measurements on the components that are connected directly to the mains. This gives the oscilloscope a floating ground, which allows the voltages in the circuit to be measured safely.

3.4 Power Control

The magnetron power control method will be implemented on the low voltage side of the supply transformer. This controller will reduce the average supply voltage of the transformer which will reduce the anode voltage and so the anode current. Figure 3.11 shows the block diagram of the control method.

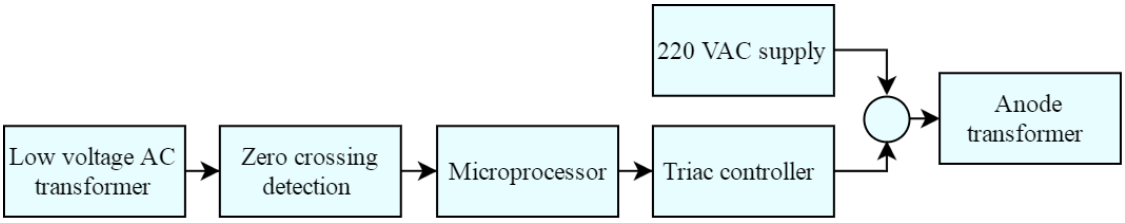


Figure 3.11: Magnetron power controller

The zero crossing detection circuit uses the voltage from the low voltage AC transformer to synchronize the microprocessor with the 50 Hz mains. This is done to ensure the trigger signals from the microprocessor are sent at the same instance in each period. When the triac controller is triggered it allows the 220 VAC_{rms} signal to be connected to the transformer.

The microprocessor is triggered by the zero crossing and then delays the triac triggering signal which blocks the supply voltage to the transformer. By doing this, the average voltage to the transformer is changed which leads to a reduced magnetron voltage and therefore lower magnetron output power. In this project this delay time is expressed as a percentage of half of the period of the 50 Hz supply voltage. A 0% delay corresponds to a 0 ms delay and means the magnetron is on at full power. A 100% delay represents a 10 ms delay and means the magnetron is off. An example of the transformer voltage supply for a 20% triac delay (2 ms) is shown in Figure 3.12.

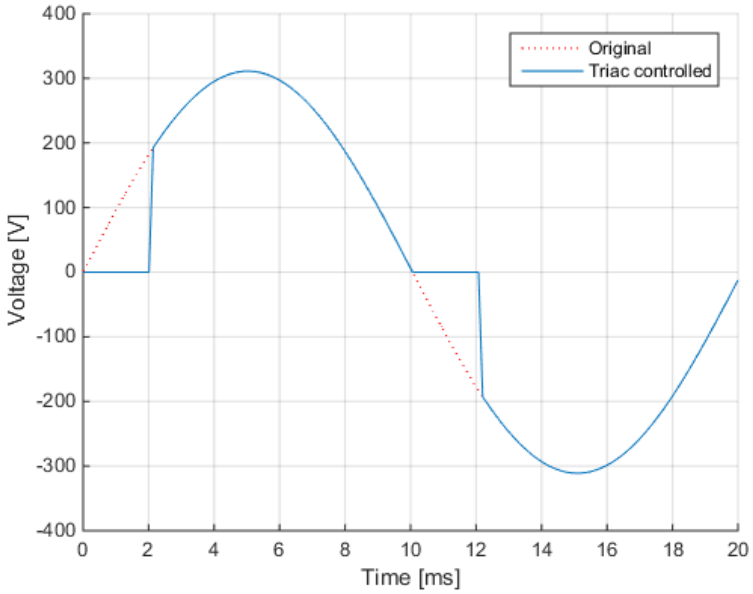


Figure 3.12: 20% delay time triac control

The software for the microwave power control was implemented with a microprocessor and serial communication to a Matlab program that handles the data processing and the control system. The software for the program is discussed in Chapter 8: Final Design.

3.4.1 Circuit Description

A circuit was designed that would interrupt the microprocessor at each zero crossing to synchronise with the main 220 VAC_{rms} voltage. This circuit is shown in Figure 3.13.

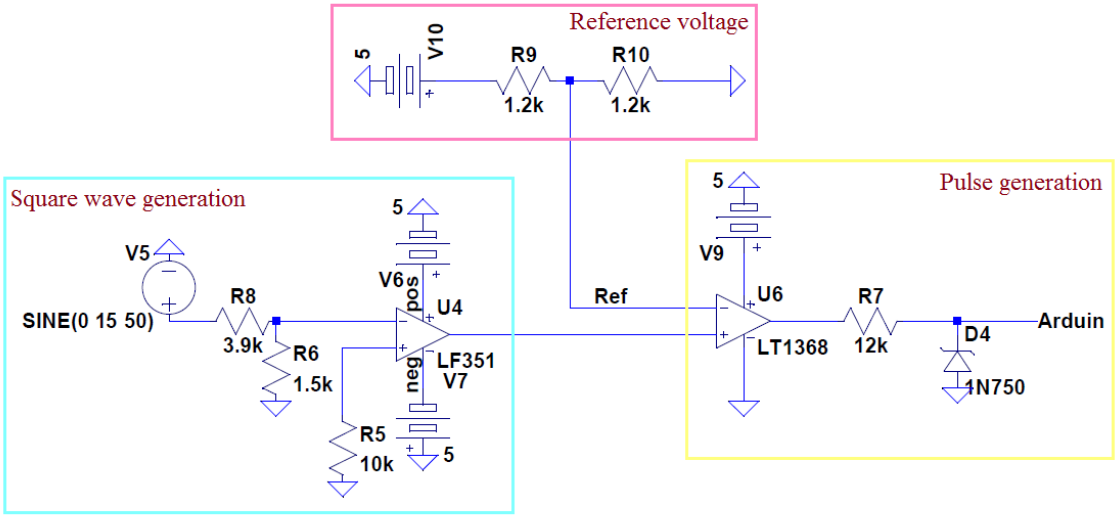


Figure 3.13: Zero-crossing detection circuit

The first part of the circuit is the square wave generator that receives a 15 VAC_{rms} voltage from a stepdown transformer. This is then sent through a voltage divider to reduce the voltage to below 5 V to protect the IC's in the circuit. The reduced voltage is then passed through a rail to rail opamp which gives out a ±5 V square wave.

The second part is a voltage divider which generates a 2.5 V reference signal for the pulse generation part of the circuit. Lastly, another rail to rail opamp is used which operates between 0 V and 5 V. The ±5 V square wave is passed to this opamp along with the reference signal which gives an output that is a square wave between 0 V and 5 V to protect the microprocessor's A/D converter. The rising and falling edges of this square wave represent the zero-crossing points of the 50 Hz input voltage and is read by the microprocessor and used to time the triac triggering.

The triac triggering works as follows: the triac switches on at a pre-set delay time after the zero crossing. By giving only a single pulse, the triac will remain on until the current has reached zero again [24]. This method of control is easily implemented for resistive loads because the voltage and current for these loads are in phase. For control of an

inductive it is necessary to send a pulse train after the initial pulse to keep the triac switched on because in inductive loads the current and voltage are out of phase. If triggered correctly, this method of pulse trains can lead to a complete absence of faults for inductive loads [24]. The transformer is a complex load and it was decided to implement this pulse train triggering to ensure the triac remains on. Figure 3.14 shows the pulse train sent for a 50 % triac delay along with the 50 Hz signal used for zero crossing detection.

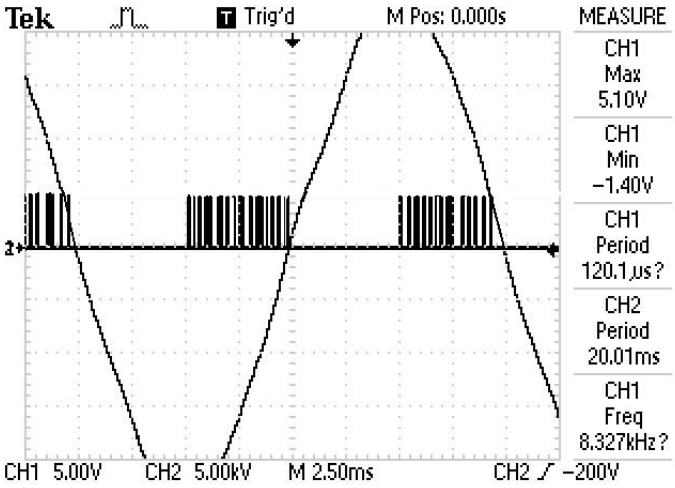


Figure 3.14: Pulse train with 50 Hz reference signal

Figure 3.15show a close-up of the pulses which has an on-off cycle every 200 µs.

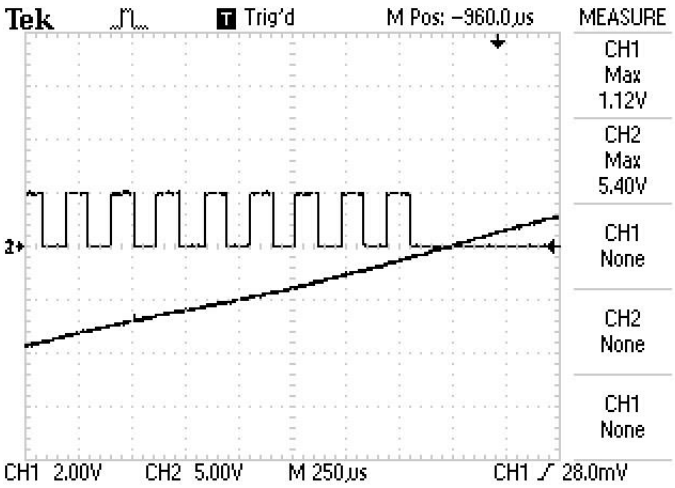


Figure 3.15: Close-up view of the pulse train

The power control circuit that was developed using a triac is shown in Figure 3.16.

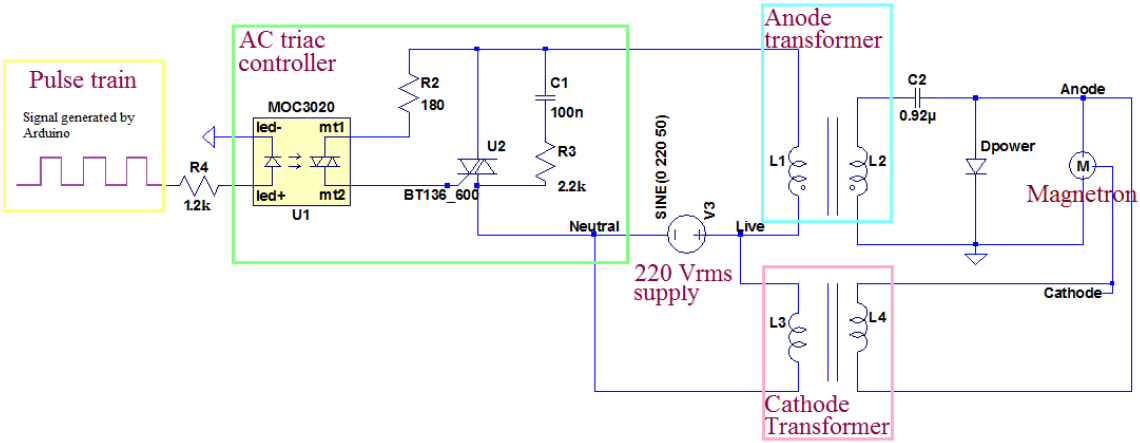


Figure 3.16: Power control circuit

The pulse train generated by the control microprocessor is sent to the MOC 3020 optic isolator in the AC control section of the circuit which in turn triggers the triac. This optic isolator is there to protect the separate sides of the circuit from any faulty voltage surges that may occur. When the triac is switched on, it connects the 220 VAC_{rms} supply to the anode transformer.

The last part of the circuit is the high voltage capacitor and a diode voltage doubler circuit which provides the high DC voltage connected to the magnetron. Usually both the anode and cathode is connected to the same transformer, but it was decided to use two separate transformers after tests showed better power control is possible when the cathode is permanently switched on.

3.4.2 Transformer Configuration Tests

With anode power control methods applied to a single transformer, as indicated in Figure 2.5, the cathode can become too cold at lower power settings. This can greatly reduce the output power of the magnetron. The developed microwave power control method was tested on a single transformer as well as two separate transformers for the anode and the cathode.

Single transformer: The first test consisted of only one transformer which was connected to both the anode and cathode. This is the standard connection of domestic microwave ovens and is shown in Figure 3.17. The triac delay time was gradually increased and the magnetron output power measured using the detector diodes.

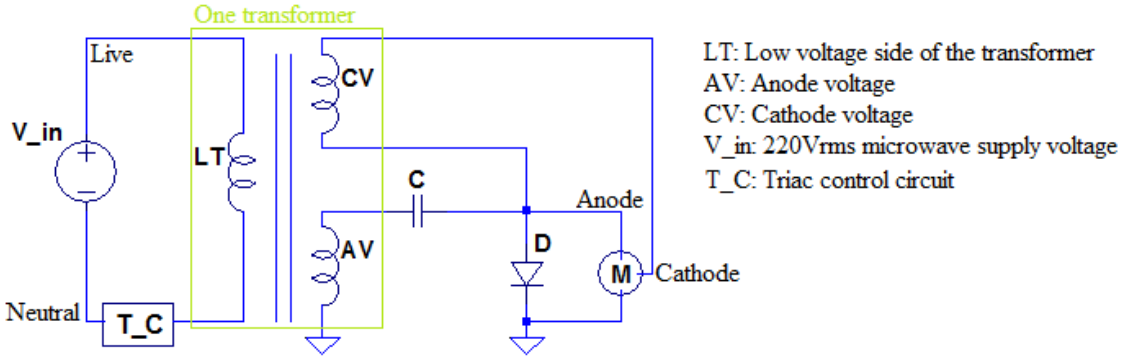


Figure 3.17: Single transformer controlled and connected to the magnetron

Cathode control: The second test consisted of two transformers - one for the anode and one for the cathode. Only the cathode voltage was controlled while the anode was kept at full voltage, and again the magnetron output power was measured for various delay times. The connection of the two transformers can be seen in Figure 3.18 .

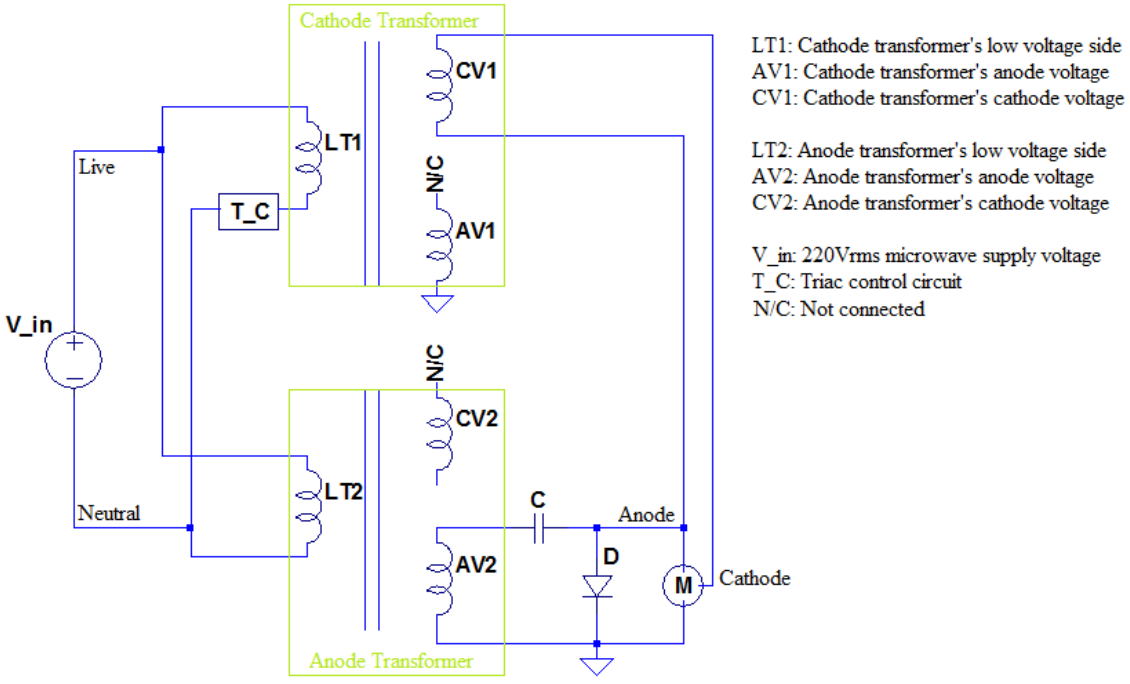


Figure 3.18: Two transformers connected to control the cathode voltage independently

Anode control: The last test moved the triac control circuit to the anode transformer, keeping the cathode at full power as seen in Figure 3.19. Again, the magnetron output power was measured for various delay times.

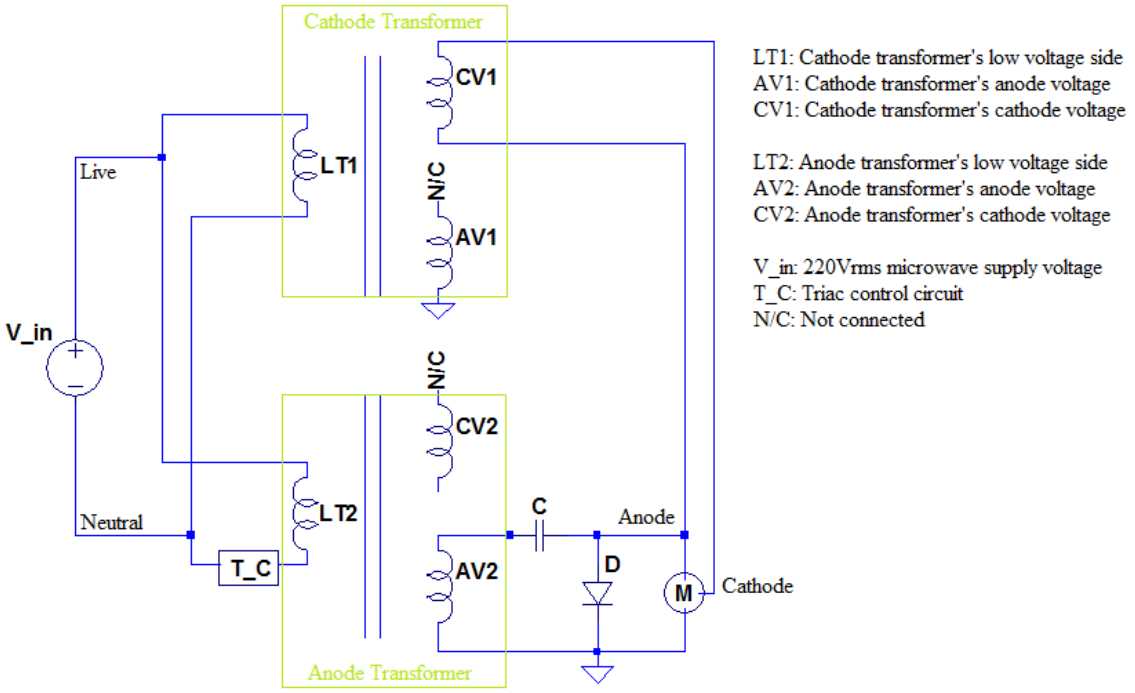


Figure 3.19: Two transformers connected to control the anode voltage independently

The measured output power of the magnetron for the different triac delay times of all three tests can be seen in Figure 3.20.

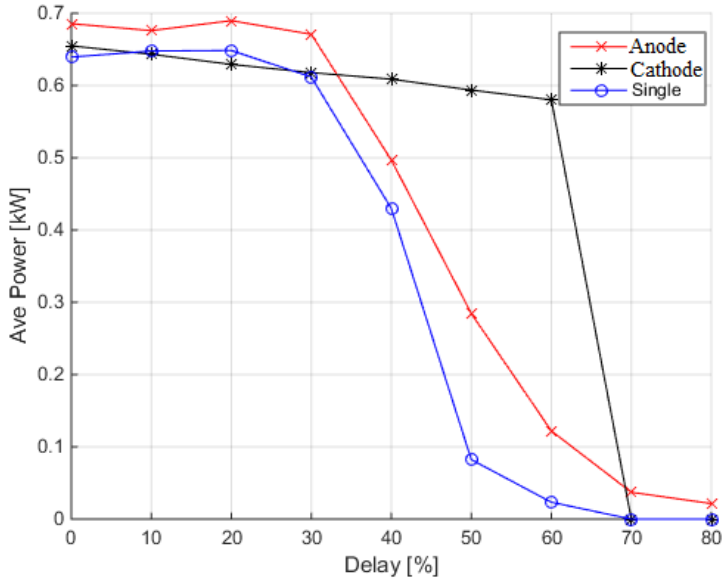


Figure 3.20: Transformer control test results

From these results it is clear that controlling the anode voltage while keeping the cathode voltage constant is the best configuration since this provides the widest linear range to control the power. This shows that the final design will need two separate transformers for proper magnetron output power control. Also note the near linear region for power output between 30% and 70%, this is where the system control will be implemented.

3.5 Relating Microwave Power to Voltage or Current

Because the dual directional coupler with the detector diodes will not be used in the final microwave oven setup alternative methods of measuring magnetron the output power were investigated.

3.5.1 Relate Anode Voltage to Power

The first method was to monitor the high anode voltage, and relate this value to the output power measured using the detector diodes. The magnetron switches on when the DC voltage from the capacitor is applied to it. Figure 3.21 shows the discharging of the high voltage capacitor along with the detector diode measurement. Channel one measured the voltage of the detector diodes and channel two measured the anode voltage.

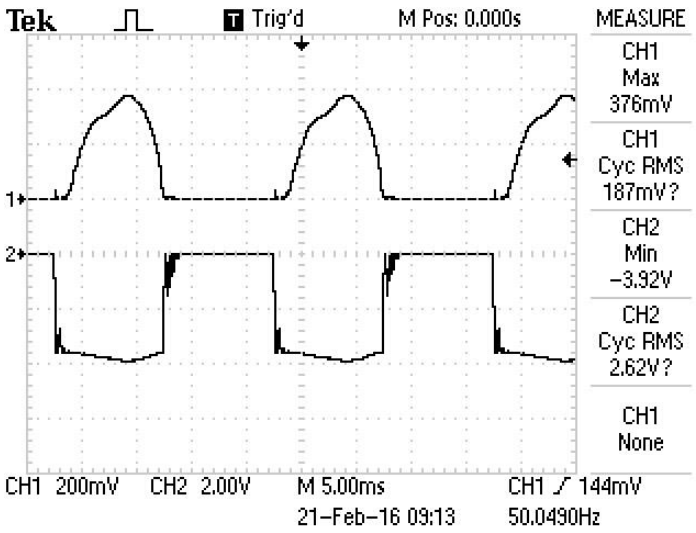


Figure 3.21: Discharging of the high voltage capacitor while the magnetron is on

The absolute minimum value of this voltage was determined in Matlab and plotted against the average magnetron output power. It was found that this value decreased as the power

decreases. Although there seemed to be a linear correlation in the 30% - 70% control region, the measurements cannot be repeated reliably. This will not be a suitable method to monitor the power. The results of these tests can be seen in Figure 3.22. Future studies could be done to improve this method.

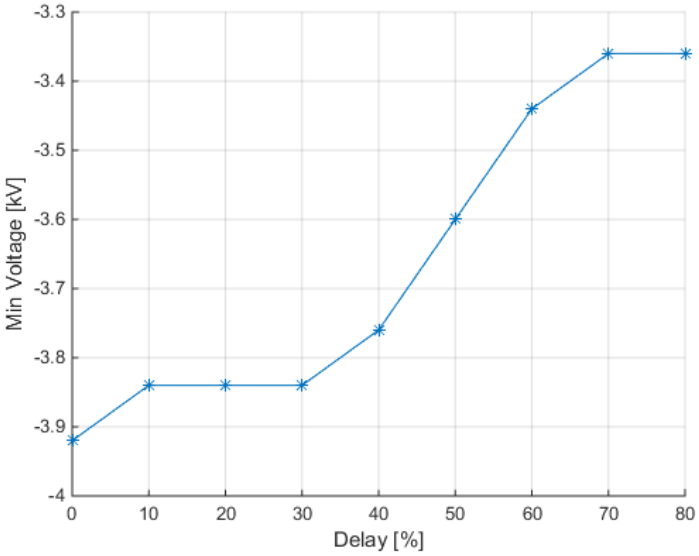


Figure 3.22: High voltage changes with delay times

3.5.2 Relate Anode Transformer Input Current and Voltage to Power

The next method for power measurement investigated the current and voltage on the primary side of the transformer and correlated those values to the magnetron output power.

Initially a split core current transformer was used, but this could not accurately measure the DC component of the current and was deemed unreliable for this project. A Hall effect sensor which allowed for both AC and DC measurements was selected. This sensor requires a 5 V supply voltage and generates a 2.5 V reference signal internally. The output of this sensor is a voltage signal around the reference voltage which can be read by the microprocessor. The Hall effect sensor is shown in Figure 3.23 measuring the transformer primary current from the triac circuit.

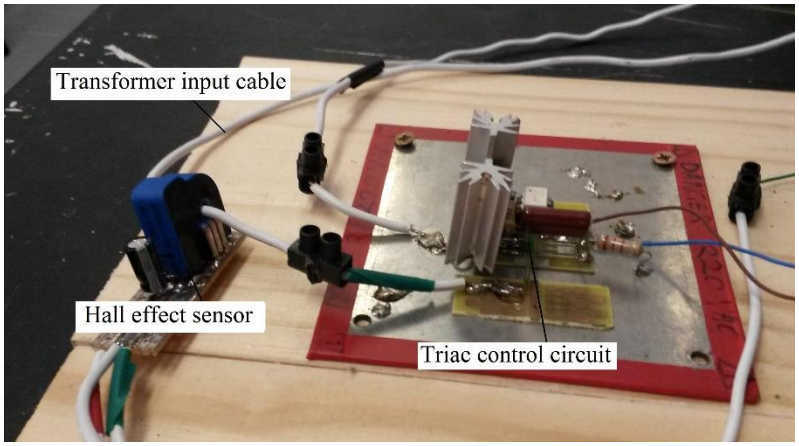


Figure 3.23: Hall effect sensor in use with the triac controller circuit

Figure 3.24 shows the measured current using both the initial and the Hall effect sensors. The measurements taken with this current transducer were proven to be correct when the power calculated using this current corresponded with the results measured using the power detector diodes.

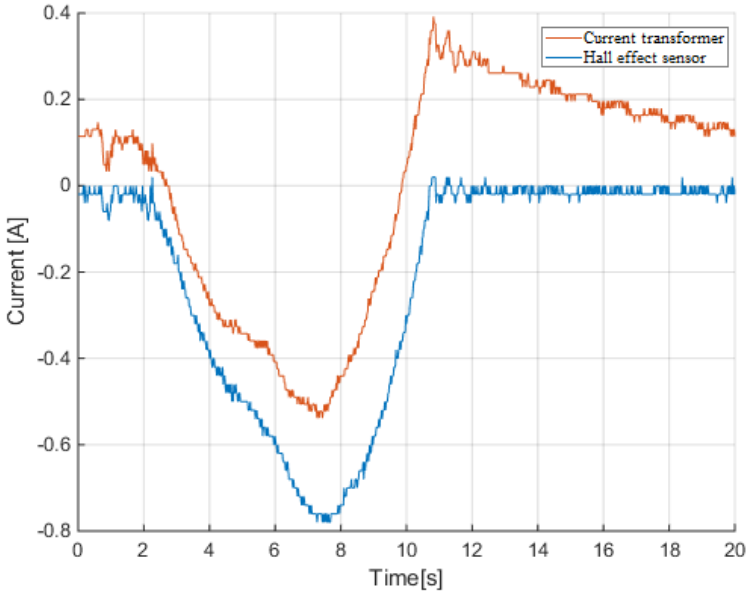


Figure 3.24: Current measured with the current transformer and the Hall effect sensor

Although this method works better than the high voltage attempt, when the measured rms current is related to the power, the initial magnetron output power (between 0% and 30% triac delay) causes duality in the measurements. A current of 4.76 A_{rms} or higher corresponds to two power measurements. This can be seen in Figure 3.25 with the triac delay values indicated.

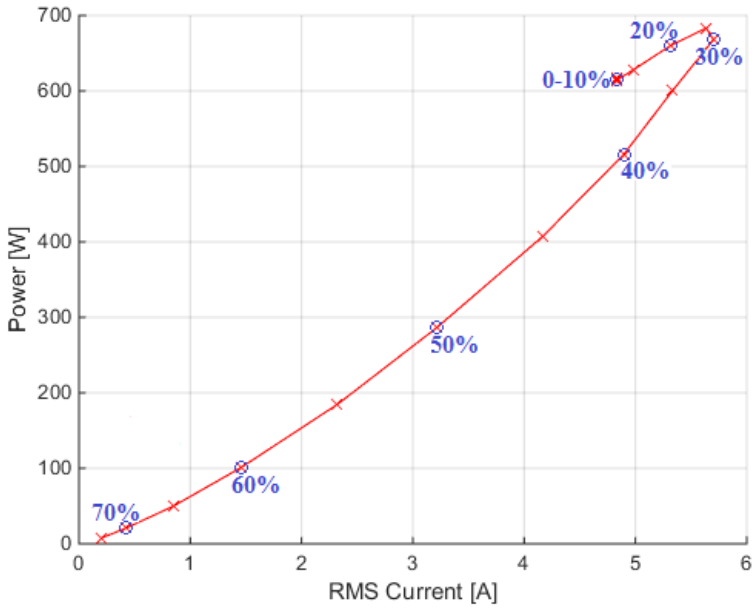


Figure 3.25: Output power versus the measured rms current

3.5.3 Measuring Without Detector Diodes

Processing the detector diode measurements can be very time consuming when multiple tests are done. This processing time can be greatly reduced by calculating the input power of the anode transformer using the measured current and the supply voltage. This input power can then be used to calculate the output power of the magnetron using the efficiency of the microwave oven. However, the supply voltage cannot be easily measured using the microprocessor therefore it was decided to simulate this voltage in Matlab.

The 220 VAC_{rms} supply was simulated for the different triac delay times and were confirmed by using an oscilloscope. The simulated voltage for each triac delay was multiplied with the corresponding current measurements which resulted in the input

power for the anode transformer. Figure 3.26 shows the simulated input voltage for 50% triac delay.

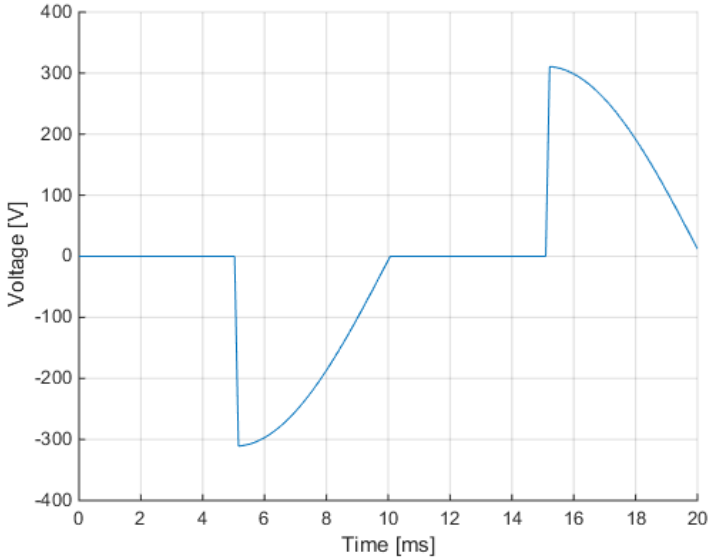


Figure 3.26: Simulated input voltage at 50% triac delay

Figure 3.27 shows the corresponding current measured using the Hall effect sensor. The sampling time for the current measurements is 20 ms which is the period of the supply voltage, 50 Hz. These measurements are also synced with the supply voltage by using the zero crossing previously described.

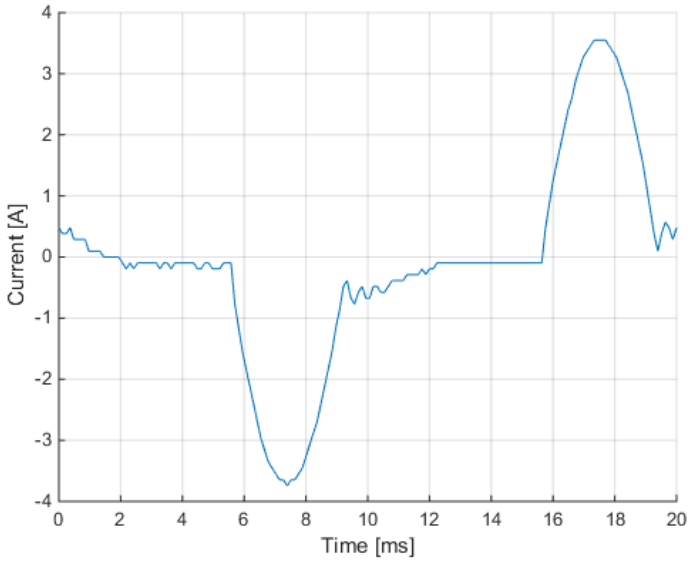


Figure 3.27: Measured current at 50% delay

The rated efficiency of the microwave can be calculated as 58.3% by using the rated input power of 1200 W and the rated output power of 700 W. This calculated efficiency is based on the assumption that the rated values are correct. The measured input power of the transformer when the magnetron is fully on is 1152.3 W and the measured output power of the magnetron is 673.9 W. This yields an efficiency of 58.5% which shows the components are working as expected.

Using this efficiency on the calculated transformer input power for each delay time, the magnetron output power was found. This method was confirmed by comparing the calculated magnetron power with the power diode measurements as seen in Figure 3.28.

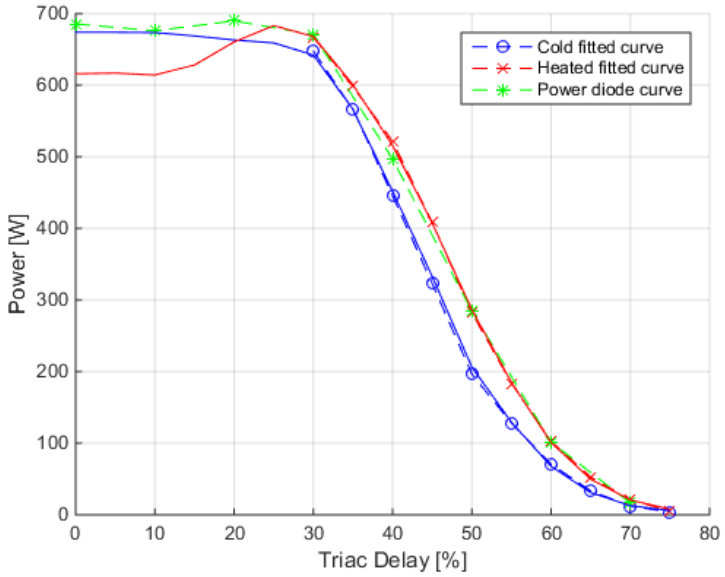


Figure 3.28: Magnetron output power calibration curves

The magnetron's output power changes with its temperature, therefore yielding heated and cold curves. This effect is referred to as thermal drift.

3.5.4 Thermal Drift Measurements

The magnetron output power was measured using the detector diodes while the temperature was monitored from a cold start at full power until thermal equilibrium was reached. The thermal drift effect on the power over time can be seen in Figure 3.29.

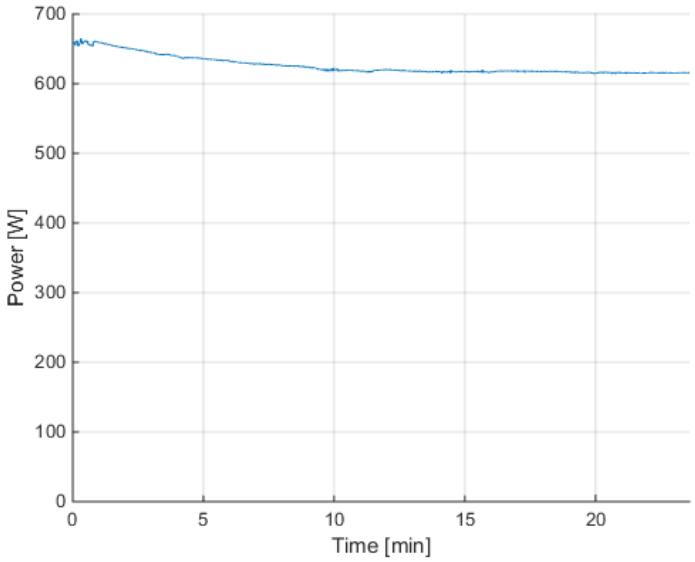


Figure 3.29: Thermal drift of the magnetron

It is important to characterize the magnetron output power in terms of its operating temperature to ensure accurate power control in the final system. These relationships were determined by first measuring the magnetron output power at increasing delay times from a cold start. These measurements were used to determine the cold calibration curves.

The magnetron was heated up to thermal equilibrium by leaving it on at full power for 30 min with a loaded cavity. After 30 min, current measurements were taken again to determine the power curves for a heated magnetron.

These tests were repeated on multiple days to account for different ambient temperatures and environmental factors that may affect the results. The measured heated and cold power curves can be seen in Figure 3.30 along with the fitted calibration curve for each case. These fitted curves are used in the control program of the final setup as discussed in Chapter 8: Final Design.

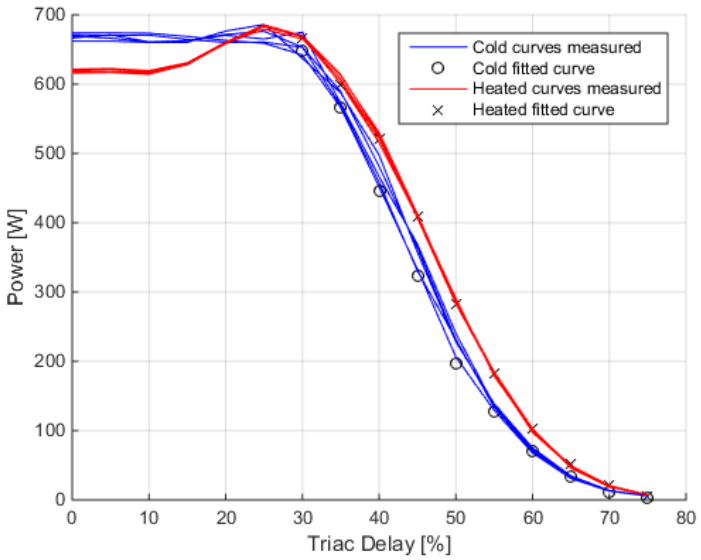


Figure 3.30: Difference in magnetron power depending on magnetron temperature.

3.6 Chapter Closing

This chapter developed a method of magnetron output power control through anode current control. This system uses a triac controller to change the input voltage of the anode transformer, thus changing the anode current and so the output power of the magnetron. A method was developed to relate the transformer input current to the magnetron output power to eliminate the need for detector diodes in the final system. The thermal drift influences on the magnetron output power was also documented.

The next chapter will discuss the selection of temperature sensors, the pump and coil to be implemented in the final system.

Chapter 4:

Component Selection

4.1 Chapter Summary

This chapter covers the selection of components that will be used in the final design. Figure 4.1 shows where this work fits into the main project development.

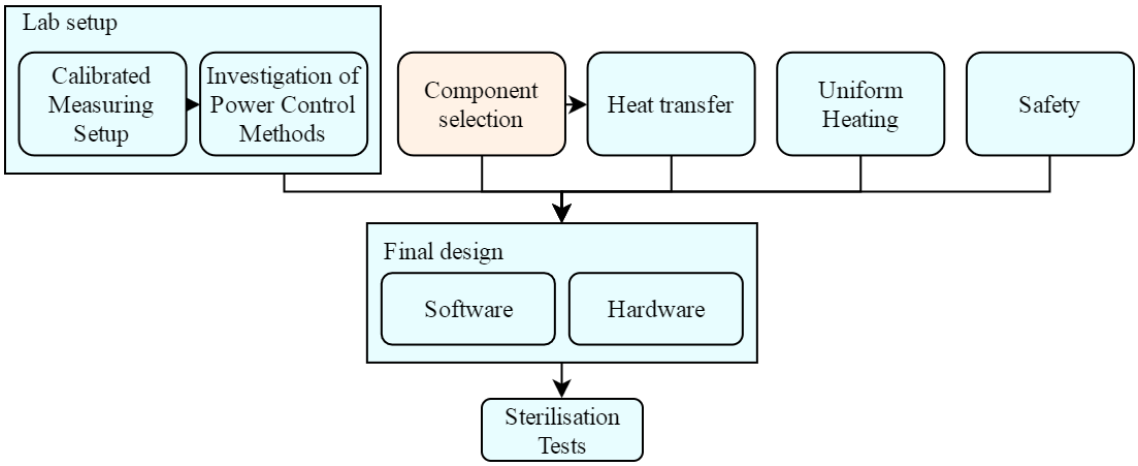


Figure 4.1: Chapter's work area in the main project overview

The flow of this chapter can be seen in Figure 4.2.

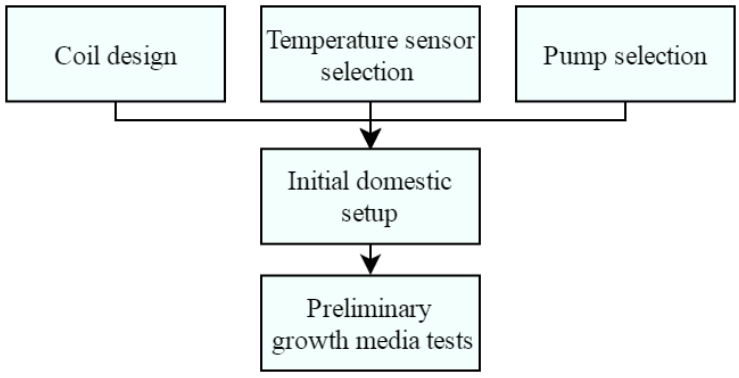


Figure 4.2: Chapter flow diagram

Pipe sizes are tested to determine the best size to use for the final coil design and temperature sensors are investigated that can operate reliably in the measurement environment surrounding the microwave oven. A pump for the final design is sourced that can supply the correct flow rate without damaging the growth media.

Finally, these components along with the developed microwave power control method are incorporated into a domestic microwave oven, which is the start of the final setup. This initial domestic setup is used to test a batch of growth media to confirm that there is no damage done to the media when using this system.

4.2 Components

4.2.1 PTFE Coiled Pipe

Glass and PTFE are often used in laboratory equipment as these materials are not reactive. This means the pipes will not chemically contaminate the fluids used during tests. Both are also non susceptible to microwaves, making both good materials for the project. The decision to use PTFE pipes was based on availability, cost and safety as they do not run the risk of potentially shattering.

The pipe sizes that were procured were all 1 m in length with inner diameters of: 1, 2, 4, 6, 8 and 12 mm respectively. Based on tests done using these, it was found that with pipes with inner diameters of 2 mm and smaller the pressure build-up in the system becomes too high. This caused back pressure to the pump and seals around the temperature sensors began to rupture. With pipe inner diameters of 8 mm and higher the pipes are not completely filled with the flow rate range. This causes air pockets in the pipes which will increase non-uniform heating of the growth media. Some of these air pockets are shown in Figure 4.3.

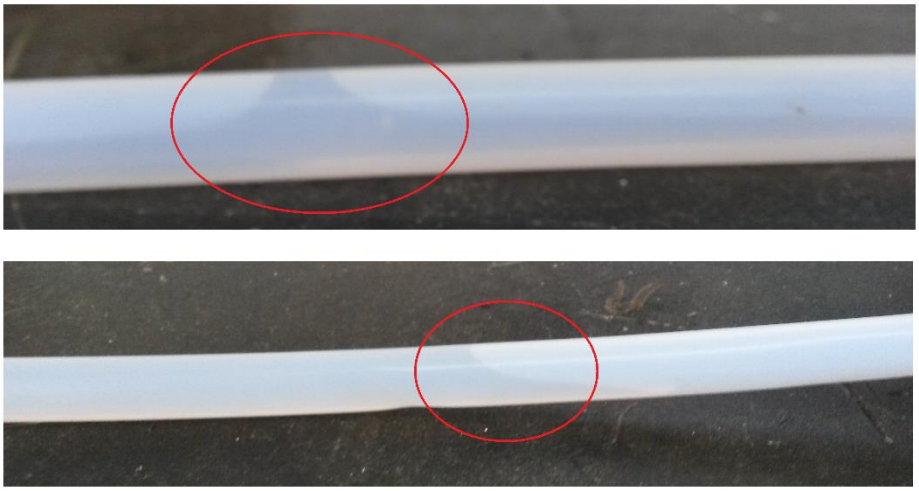


Figure 4.3: Air pockets present at in larger diameter pipes

The 4 mm and 6 mm pipes both were large enough to not cause back pressure and small enough to not cause air pockets within the pipes. A coiled pipe was designed to be placed

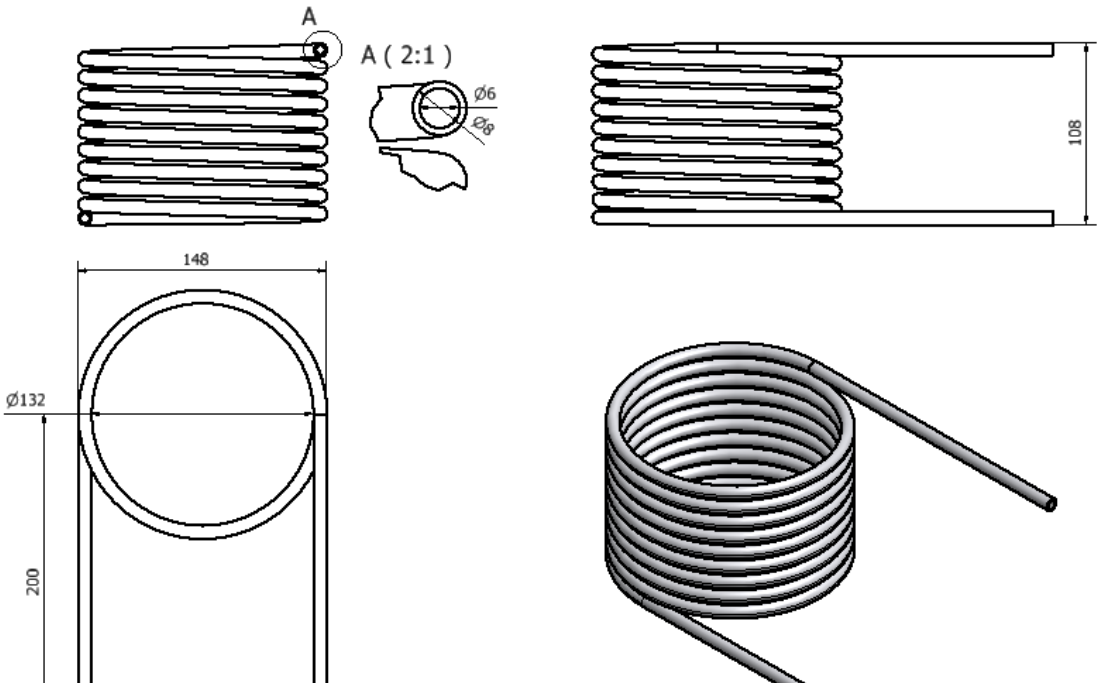


Figure 4.4: Coil dimensions

in the centre of the domestic microwave oven using 6 mm inner diameter pipe. Figure 4.4 shows the designed coil which was turned using 5 m PTFE pipe.

4.2.2 Temperature Sensors

For the final system to work, the temperature sensors have to be accurate to within 1 °C and reliable in the measurement environment around the microwave oven. The temperature sensors that were initially used were low-power linear active thermistor IC's (MCP9700-E/TO). During the first temperature tests it was found that the sensors produced unreliable readings as these sensors were used to measure mV signals in an area around the high voltage transformers used to generate kV signals.

Various methods were tried to eliminate the interference on the sensor reading, including moving the sensors, replacing the cables with shielded twisted pair cables, insulating the sensor with aluminium tape and only measuring in the off cycle of the magnetron. These methods helped reduce the interference, but did not eliminate the problem and the sensors were deemed unreliable for this application. An example of the output signal with interference and with reduced interference is shown in Figure 4.5.

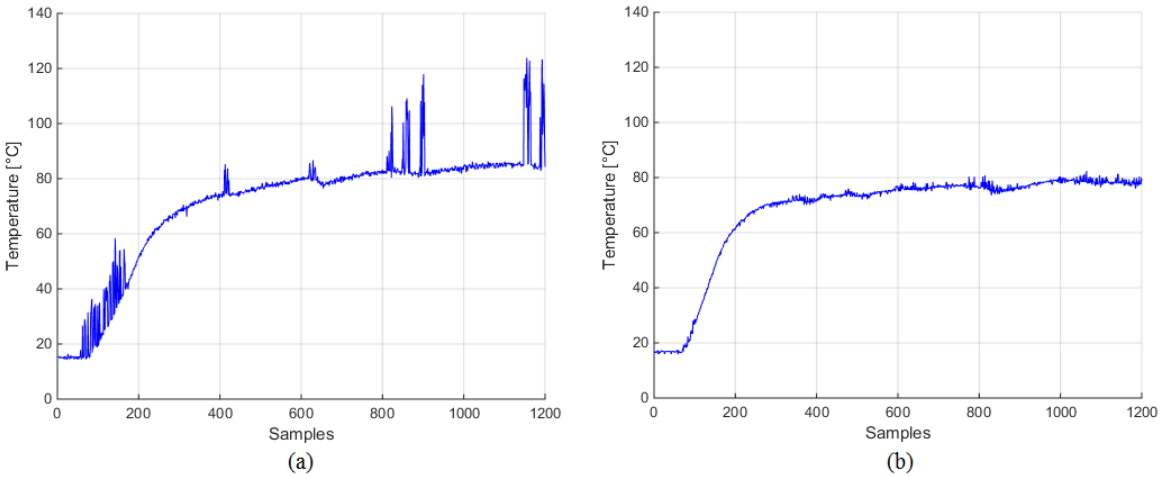


Figure 4.5: (a) Original sensor output; (b) Sensor output after adjustments

These temperature sensors were replaced with PT100 probes which are resistance temperature sensors. These probes have internal structures most commonly made of platinum, which expands and contracts with changes in temperature. Platinum probes have a linear resistance to temperature relationship over a wide range: -200 °C to

800 °C [25]. The resistance of the probe changes with temperature and since it has no active electronics inside and is better shielded, the probe is less susceptible to interference around the microwave. The probe's wires are connected in a measuring circuit as discussed in Chapter 8: Final Design, and the output voltage is measure using the microprocessor.

The PT100 probes have general resistance vs temperature tables that can be used to measure the temperature. Since the probes are used in a circuit that gives out a voltage to the microprocessor, it was thought best to physically calibrate the probes with their measuring circuits in order to get a direct relationship between temperature and voltage which incorporates any minor differences in the circuit component values and gains that may affect the output voltage.

This calibration process was done by using a trusted digital thermometer as a reference and ice slush as the 0 °C point. The ice slush was slowly heated using a kettle while the temperature and the voltage was measured. These measurements were taken at intermittent times up to boiling point. The calibration curves for both the inlet and the outlet sensors can be seen in Figure 4.6 with the measured data points.

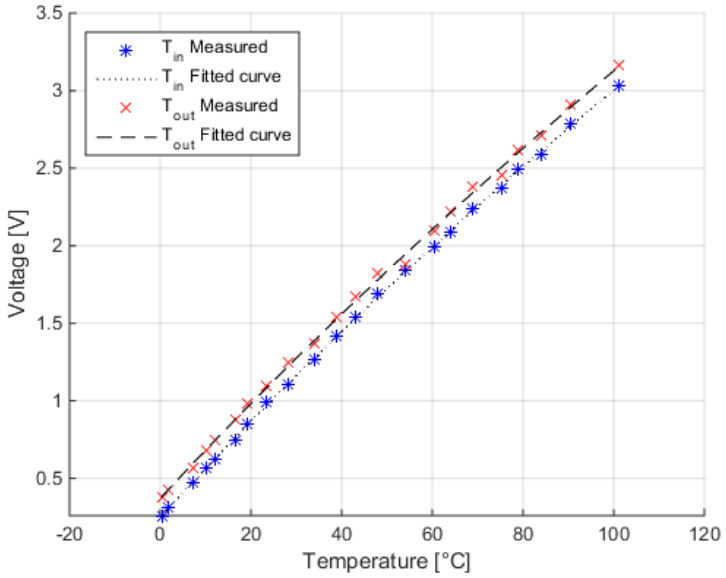


Figure 4.6: Calibration curve of PT100 probes

The error between the calibrated curve and the measured values were calculated to confirm the accuracy of the sensors. The project specifications stated that the temperature should be accurate to within 1 °C. Figure 4.7 shows the calculated errors for the two PT100 probes used in this project.

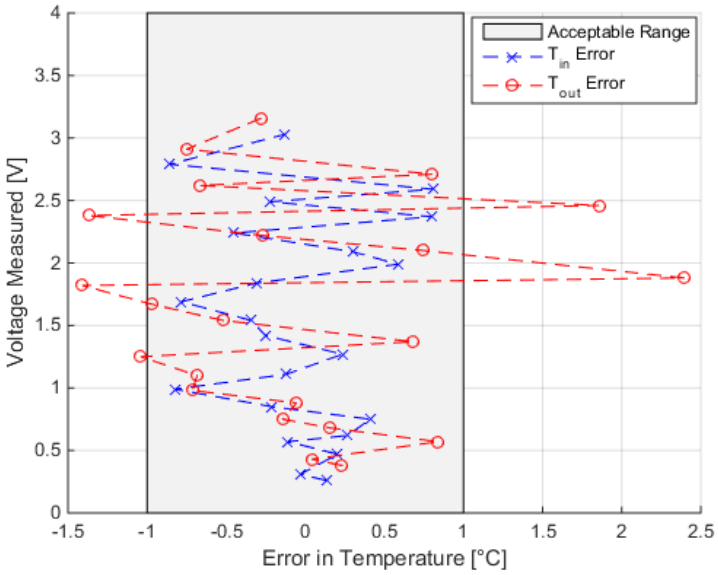


Figure 4.7: Temperature errors of PT100 probes

The shaded area indicated the acceptable ±1 °C range for this project. The few points outside this range could be due to measurement errors during tests.

An example of temperature measurements taken with these probes is shown below in Figure 4.8.

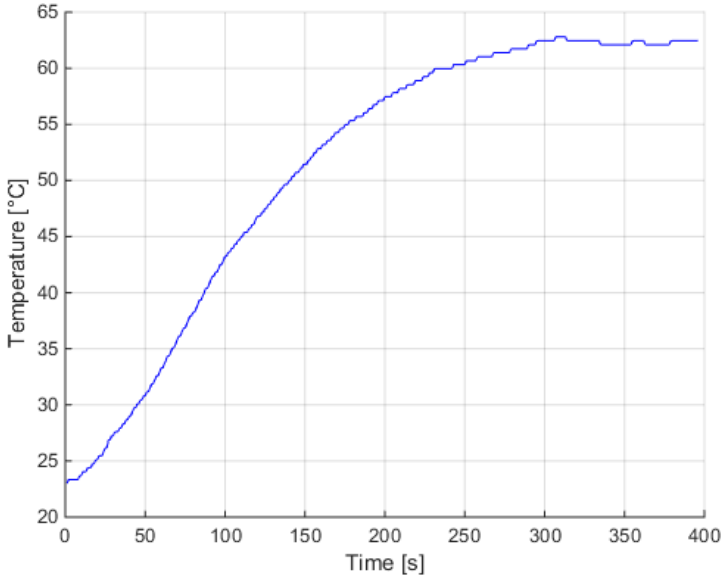


Figure 4.8: Typical temperatures measurements with the PT-100 probes

4.2.3 Pump

This system requires a pump that can be controlled along with the rest of the rest of the components. The pump should not damage the nutrients in the growth media. As an example, the friction in a centrifugal pump is too high and will damage the media.

Generally, peristaltic pumps are used in biochemical applications and, although these are not continuous flow pumps, they do have significantly less friction. These pumps, especially for the low flow rate required for this project, are expensive. An affordable one was sourced, but this pump is designed as a dosing pump for fish tanks and not for continuous use so the pump life will be reduced. For use in this project it will be sufficient, but a replacement should be found or designed if the project is further developed into a commercialised product.

The flow rate was measured manually by measuring the time it took to fill a 100 ml beaker. These measurements were done multiple times to ensure the flow rate is correct before the tests began. The relationship between the flowrate and the pwm value is shown in Figure 4.9.

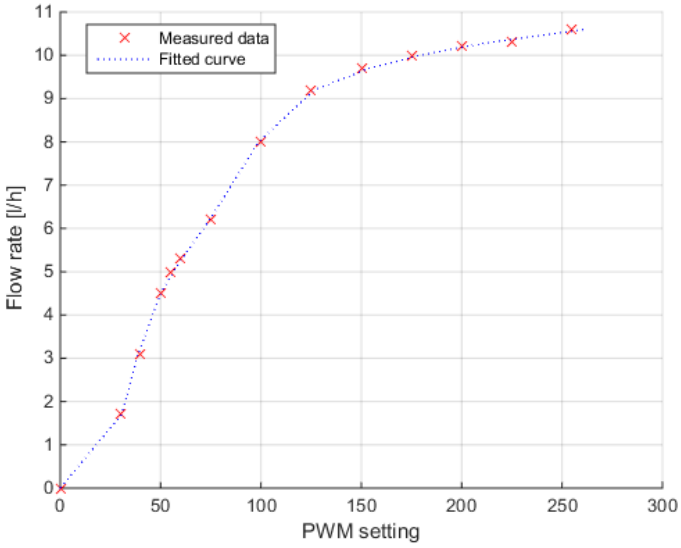


Figure 4.9: Pump flow rate for different pwm settings

4.3 Domestic Oven Setup

Domestic microwave ovens were procured and modified to develop the final system. One of the ovens was stripped for components that will be used to develop the power control method. The other was modified to allow continuous flow through the microwave cavity. This domestic microwave oven setup will be used to finalise the control system for the project.

After the microwave power control for the system was finalised using the calibrated measurement setup, the power control circuit and components were moved to the domestic oven setup for system finalisation and control system implementation. Figure 4.10 shows the coil fixed inside the microwave cavity.

Figure 4.11: Coil placed inside the microwave oven

Figure 4.11 shows the microwave modified to allow the PT100 probes to be added.

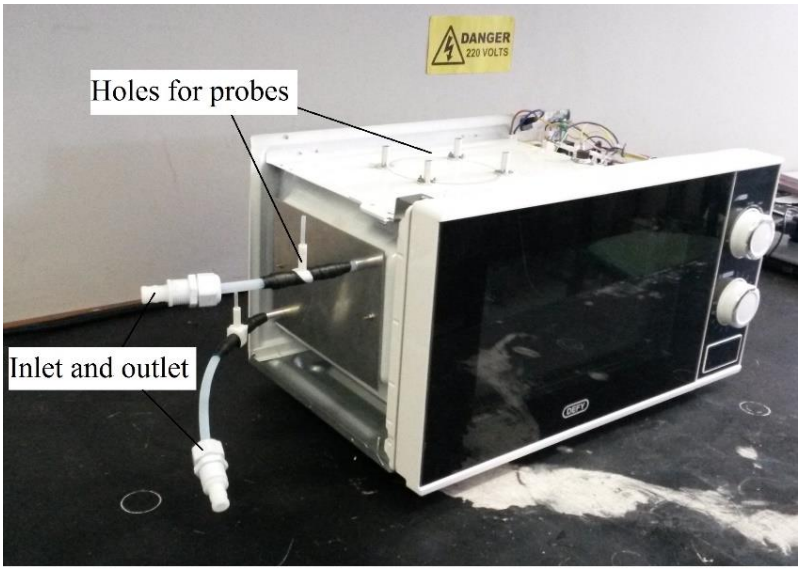


Figure 4.10: Microwave oven and coil modified for temperature probes

Figure 4.12 shows the pump and PT100 probes added to this setup.

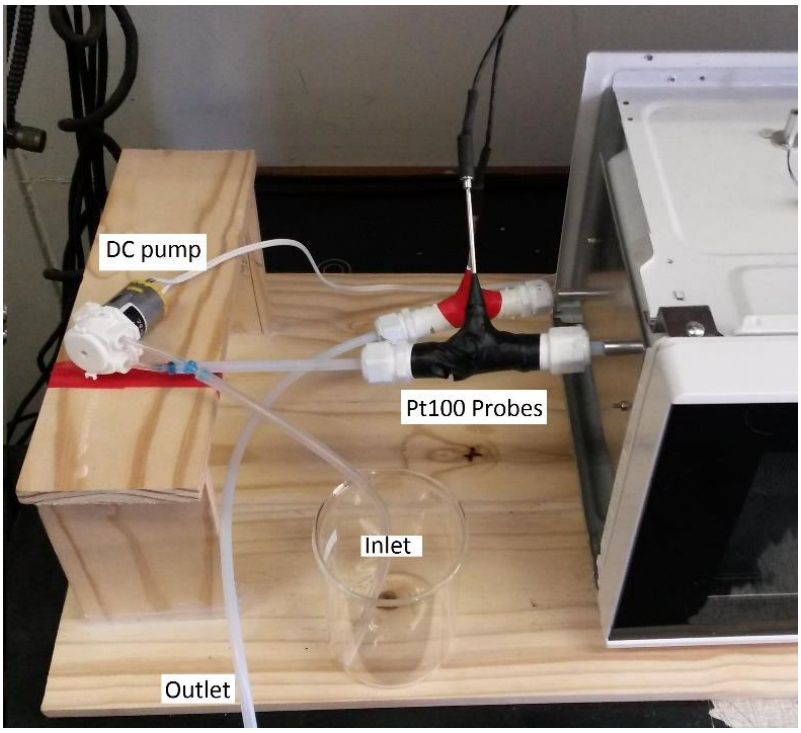


Figure 4.12: Pump setup with the inlet and outlet PT100 probes

4.4 Preliminary Tests

A batch of sterile media was received from the Biochemistry Department to test if there are any severe effects on the media. There was a concern that the microwaves may caramelise the sugars in the media. This can be seen visually as the media will turn darker in colour.

The 800 ml batch was divided into 5 samples of 160 ml each. One sample was kept unused as a reference point. The first sample was microwaved at 700 W for 2 min, reaching a final temperature of 82 °C with no discolouration observed.

The second sample was microwaved for 10 min at 300 W and the third for 20 min at 400 W. Even though both these samples started boiling neither turned darker. The last sample was microwaved at 450 W for 30 min, boiling for most of the time. This is the only sample with mild discolouration. This will set the maximum exposure time for the media at 30 min. Figure 4.13 shows the four used samples compared to the unused sample.

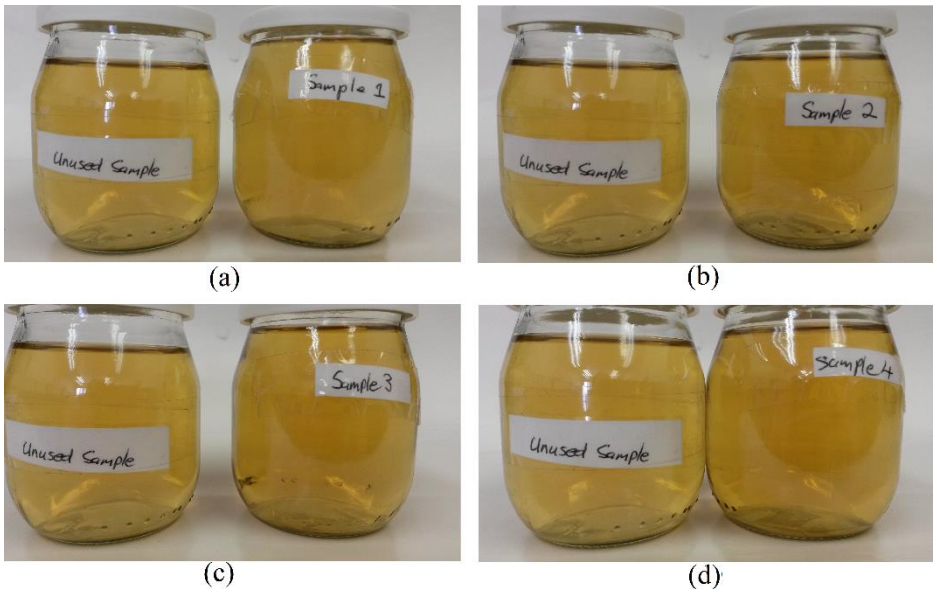


Figure 4.13: Colour comparison of: (a) Sample 1; (b) Sample 2; (c) Sample 3; (d) Sample 4

4.5 Chapter Closing

This chapter discussed the coil to be used in the modified microwave oven and the selection of temperature sensors as well as the pump to be used in the final setup. The power control method previously developed and the selected components were assembled in a domestic microwave oven setup, which is the starting point of the final system. Preliminary tests were done on growth media and the maximum exposure time for the media was determined to be 30 min. The next chapter will discuss uniform heating within a microwave oven.

Chapter 5:

Uniform Heating Within a Microwave Cavity

5.1 Chapter Summary

This chapter will discuss the problem of standing waves that cause non-uniform heating inside a microwave cavity. A mode stirrer is implemented in the cavity to disperse these waves and improve uniform heating. The effect of the mode stirrer is visualised with two methods: neon lightbulbs and thermal imaging. The work discussed in this chapter fits into the overall project as indicated in Figure 5.1.

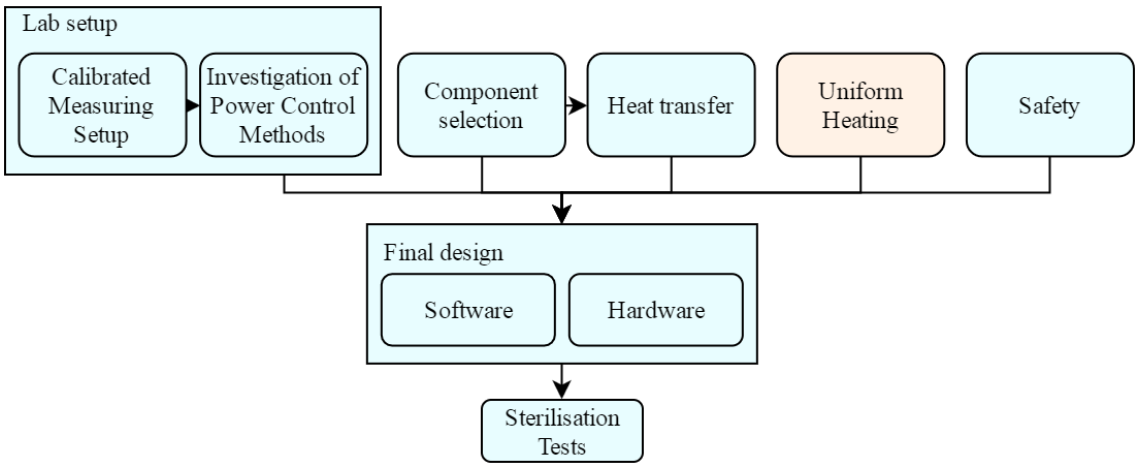


Figure 5.1: Work discussed forms part of the indicated overall project development

5.2 Standing Waves

A problem uncovered during initial tests in the calibrated measurement setup was localised boiling within the pipes. The EM-field waves inside the cavity form standing waves and these stationary nodes and antinodes cause non-uniform heating [1]. Figure 5.2 shows EM-field waves propagating along a central axis with the nodes and antinodes indicated.

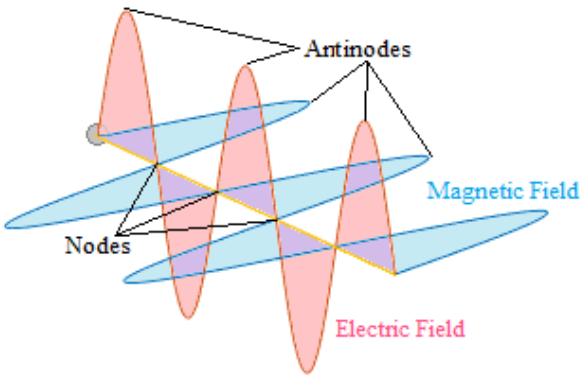


Figure 5.2: EM-field wave with nodes and antinodes

The antinodes are points of maximum energy and the nodes have minimum energy. This difference in energy distribution causes boiling at the antinodes and very little heating takes place at the nodes. This is undesirable because the non-uniform heating that occurs could cause some of the media to remain unsterilised.

As a possible solution a mode stirrer will be implemented in the modified domestic oven setup. The mode stirrer is a non-symmetric piece of metal that is rotated inside the cavity. The waves are continuously reflected in different directions so permanent standing waves cannot form. This will lead to heating which is more uniform.

Literature is inconclusive in terms of the precise extent to which a mode stirrer improves uniform heating. Electromagnetic simulations of cavities with mode stirrers have not been extensively done to provide more insight into this. Qualitative evaluations using thermal paper have been performed which shows some improvement in uniformity, but does not provide complete uniform heat distribution [26].

5.3 Visualising Standing Waves

The effect of the mode stirrer was shown with a simple experiment using 50 neon lightbulbs placed in a star pattern on a polystyrene sheet. These lightbulbs were placed in the microwave oven with and without a mode stirrer. It was found that without the mode stirrer only a few lights would be switched on and these few lights remained on. With the mode stirrer, the waves move continuously and almost all the lights turn on as shown in Figure 5.3.

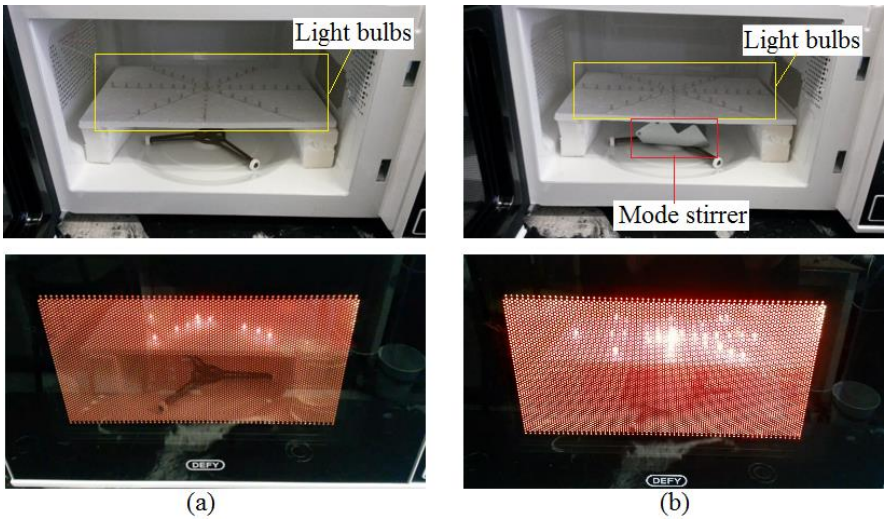


Figure 5.3: (a) Neon lights without mode stirrer; (b) Neon lights with mode stirrer

The red light seen in this image indicates where the standing waves are. Without the mode stirrer these waves remain in the same position and cause non-uniform heating. With a mode stirrer the waves are constantly reflected which leads to more lights being turned on. This shows that heating with a mode stirrer will be more uniform.

Thermal imaging was also used to get visual confirmation of improved uniform heating. This was achieved by placing a tube of cold water in a domestic microwave oven for 10 seconds without a mode stirrer. The thermal image was captured as soon as the time passed. The experiment was then repeated with a mode stirrer. This resulted was more uniform heating with the mode stirrer as see in Figure 5.4.

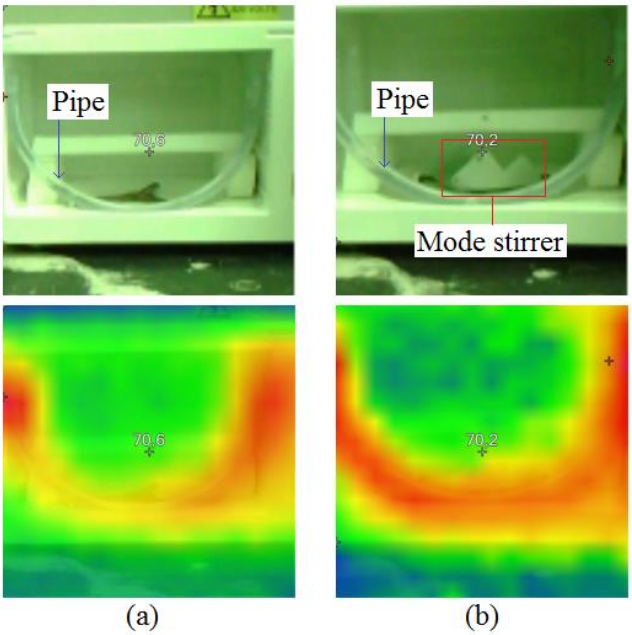


Figure 5.4: Thermal images: (a) Without mode stirrer; (b) With mode stirrer

This image shows that without a mode stirrer the tube of water gradually heats from the sides, but very little in the middle. When the mode stirrer is added, the reflected waves can be more evenly absorbed by the entire tube of water leading to more uniform heating.

5.4 Chapter Closing

This chapter discussed the problem of non-uniform heating caused by standing waves and showed how the implementation of a mode stirrer reduces the effect of these waves. The following chapter will discuss the heat transfer calculations for the project.

Chapter 6:

Heat Transfer

6.1 Chapter Summary

This chapter discusses the heat transfer of the system and fits into the overall project as indicated in Figure 6.1.

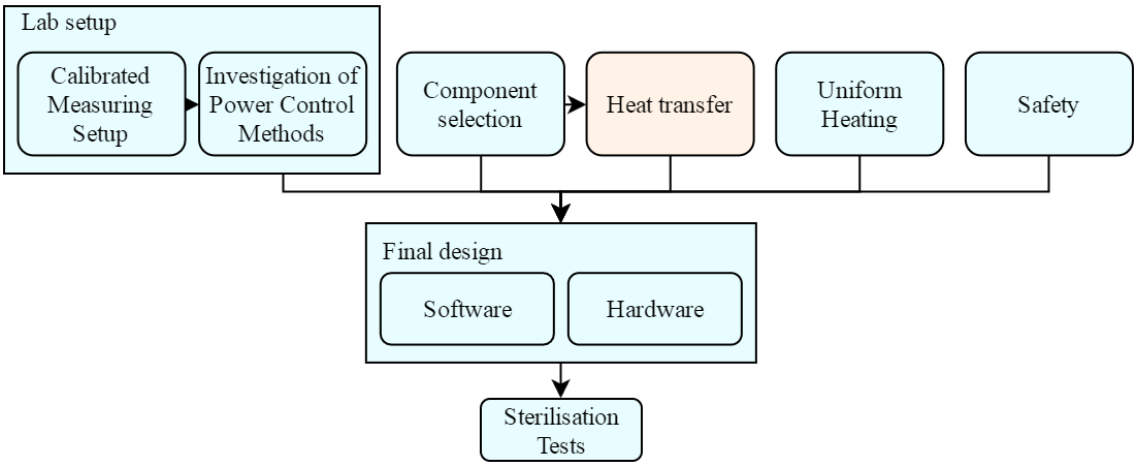


Figure 6.1: Heat transfer as part of the overall project.

The maximum and minimum power that can be absorbed by the microwave load is calculated in this chapter. The heat transfer gradients for different flow rates are also simulated and compared to measured data. Lastly, the microwave setup's maximum temperatures achievable is compared to the temperatures reached when using a steam chamber instead, for the same volume and flow rates.

6.2 Microwave Equations

The system being simulated has the fluid flowing through the PTFE coil inside the microwave cavity. The heat transfer within the microwave cavity was simulated to predict the achievable exit temperatures with known inlet temperatures and microwave power into the cavity. This system is shown in Figure 6.2.

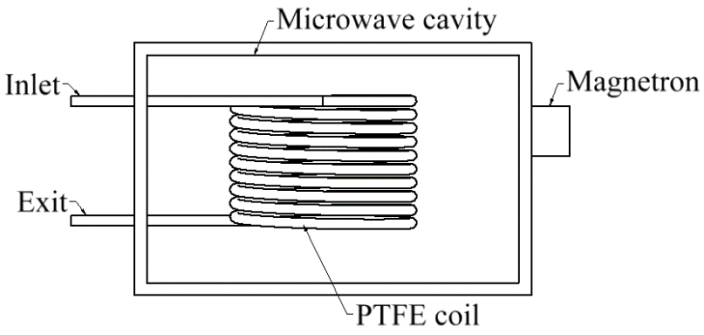


Figure 6.2: Microwave oven system

First, the absorption limits of the system have to be determined. The minimum power that the load has to absorb to ensure the reflected power does not damage the magnetron is:

$$P_{min} = \frac{1000V_{tube}}{250} \qquad \textbf{6-1}$$

Where V_{tube} [ml] is the volume of the tube.

The maximum power that the load is able to absorb is calculated using the electric field strength. Since the electric field is constantly changing, the exact value differs with time and location within the chamber.

An estimated maximum electric field, E_{max} [V/m], can be found as:

$$E_{max} = \sqrt{\frac{2P_{rated}\mu_0 c}{A_{cav}}} \qquad \textbf{6-2}$$

With:

P_{rated} is the rated output power of the microwave [W]

μ_0 is the permeability of free space: $4\pi\times10^{-7}$ [H/m]

c is the speed of light: 3×10^{8} [m/s]

A_{cav} is the area enclosed by the cavity [m^2]

The rms value of the electric field is used to estimate the maximum power limit more accurately.

$$E_{rms} = \frac{E_{max}}{\sqrt{2}} \qquad \textbf{6-3}$$

The maximum power the load can absorb is then calculated by [27]:

$$P_{max} = 2\pi f \varepsilon_0 \varepsilon'' {E_{rms}}^2 V_{tube} \qquad \textbf{6-4}$$

Where: f is the operating frequency of the magnetron [Hz]

ε_0 is the permittivity of free space: 8.85418782×10^{-12} [F/m]

ε'' is the dielectric loss in the load

When simulating the heat transfer in the microwave oven, the power absorbed by the load, P_{abs} [W], can be calculated as [28]:

$$P_{abs} = \dot{m} C_p \Delta T = \dot{V} \rho C_p \Delta T \qquad \textbf{6-5}$$

With: C_p is the specific heat [J/kg°C]

$\dot{m}$ is the mass flow rate [kg/s]

$\dot{V}$ is the volumetric flow rate [m^3/s]

ΔT is the difference between the inlet, T_i, and exit, T_e, temperature [°C]

Aluminium cavities can absorb up to 10% of the microwave power [28]. Therefore the adjusted power absorbed by the load is:

$$P_{adj} = 0.9P_{abs}$$

6-6

It is assumed that the absorbed power, P_{abs}, is the power into the microwave oven cavity unless this value exceeds the maximum power the load can absorb.

The maximum temperatures reached per flow rate can be calculated by solving eq. 6-5 for the exit temperature using the adjusted absorbed power:

$$T_e = \frac{P_{adj}}{\dot{V}\rho C_p} + T_i$$

6-7

6.3 Steam Chamber Equations

The maximum temperatures reached with the microwave system have to be compared to the maximum temperatures that could be reached with alternative heating systems. A steam chamber with the exact same coil and flow rates was simulated for this comparison.

In standard flow in pipes, the Reynolds number, *Re*, is a dimensionless quantity that indicates the transition from laminar to turbulent flow and is defined as [28]:

$$Re = \frac{\rho(\frac{\dot{V}}{A_x})D_{tube}}{\eta}$$

6-8

With: $\dot{V}$ is the flow rate [m^3/s]

ρ is the density [kg/ m^3]

η is the dynamic viscosity [kg/m·s]

A_x is the cross sectional area of the tube [m^2]

D_{tube} is the inner diameter of the tube [m]

The secondary flow in coiled pipes enhances heat transfer due to maximum velocity being pushed out from the centre across most of the tube by centrifugal force [28]. The Dean

number, *De*, is a dimensionless variable that indicates laminar flow in coiled pipes. Values exceeding 100 are suitable for heating of fluids in a microwave [11].

$$De = R_e \sqrt{\frac{D_{tube}}{D_{coil}}} \tag{6-9}$$

D_{coil} is the coil diameter [m].

The Nusselt number, *Nu*, is the dimensionless parameter used to indicate the relationship of conductive and convective heat transfer. For laminar flow in a coiled pipe, the Nusselt number is given by [29]:

$$Nu = (2.153 + 0.318De^{0.318})Pr^{0.177} \tag{6-10}$$

The Prandtl number, *Pr*, is a dimensionless fluid property relating to the boundary layer and type of fluid.

The convective heat transfer coefficient, *h* [W/m^2°C], is calculated as [29]:

$$h = \frac{k}{D_{tube}} Nu \tag{6-11}$$

k is the thermal conductivity [W/m°C].

The absorbed power in a system, P_{abs}, can also be defined as [30]:

$$P_{abs} = hA_s \Delta T_{lm} \tag{6-12}$$

Where ΔT_{lm} is the log mean temperature [°C] calculated as:

$$\Delta T_{lm} = \frac{T_i - T_e}{ln[(T_s - T_e)/(T_s - T_i)]} \tag{6-13}$$

T_s is the temperature of the outside surface of the tube which is assumed to be the temperature of the steam chamber.

Finally the exit temperature of the system can be calculated by equating eq. 6-5 and eq. 6-12 and solving for T_e, which yields:

$$T_e = T_s - (T_s - T_i)e^{\left(\frac{-hA_s}{\dot{V}\rho C_p}\right)} \quad \textbf{6-14}$$

All the fluid properties were evaluated at the bulk temperature, T_b [°C]:

$$T_b = \frac{T_{eTarget} + T_i}{2} \quad \textbf{6-15}$$

Where $T_{eTarget}$ is the target exit temperature of the system.

6.4 Calculations and Results

6.4.1 System Values

An inlet temperature of 18 °C and a target exit temperature of 100 °C was selected for these simulations. This leads to a bulk temperature of 59 °C. Table 6.1 lists all the fluid properties of water used in the calculations.

Table 6.1: Fluid properties of water at 59 °C

Property	**Value**	**Reference**
Density, ρ	983.3 kg/m^3	[30]
Specific heat, c_p	4185 J/kg°C	[30]
Thermal conductivity, k	0.654 W/m°C	[30]
Dynamic Viscosity, μ	0.467 ×10^{-3} kg/ms	[30]
Prandtl number, Pr	2.99	[30]
Dielectric loss, ε''	67.85	[31]

A range of flow rates were evaluated to find the maximum temperatures that the microwave system can reach using eq.6-5. Each of these flow rates was also simulated for different input powers to determine a relationship between temperature rise and power. These values, as well as the dimensions and areas of the coil and the cavity, are given in Table 6.2.

Table 6.2: System variables for heat transfer calculations

Variable	**Value**
Tube inner diameter, D_{tube}	0.006 m
Coil diameter, D_{coil}	0.132 m
Tube volume, V_{tube}	141.37 ml
Tube cross sectional area, A_x	0.0942 m^2
Cavity surface area, A_{cav}	0.3484 m^2
Magnetron frequency, f	2.45 GHz
Flow rate range, $\dot{V}$	[3.5; 5; 6; 7; 10; 11.2] l/h
Input power range, P_{abs}	[100; 200; 300; 400; 500; 600; 700] W

6.4.2 Flow Inside the Coil

The Deans numbers had to be calculated using eq. 6-9 to ensure that the flow is laminar for all flow rates tested. The results in Table 6.3 shows that all the flow rates achieve Dean numbers above 100 except for the lowest flow rate. Although this Dean number is not above 100, it is close enough to not cause any significant problems.

Table 6.3: Calculated Dean numbers for different flow rates

Flow rate [l/h]	3.5	5	6	7	10	11.2
De	92.6	132.3	158.7	185.2	264.6	296.4

6.4.3 Microwave Calculations

Using the maximum rated output power of the magnetron, 700 W, the minimum and maximum absorbed power of the load is calculated using eq. 6-1 and 6-4 respectively. This yields P_{min} = 565.5 W and P_{max} = 990.1 W. From this it can be concluded that all the available power in the cavity will be absorbed by the load and the magnetron will not be damaged by any reflected power.

The maximum exit temperature for different input powers for each flow rate was calculated using eq. 6-7. These tests were also physically done using the microwave setup with power control. By comparing the calculated and measured values, it was found that there is a maximum error of 7% between the simulated and measured values. These results can be seen in Figure 6.3.

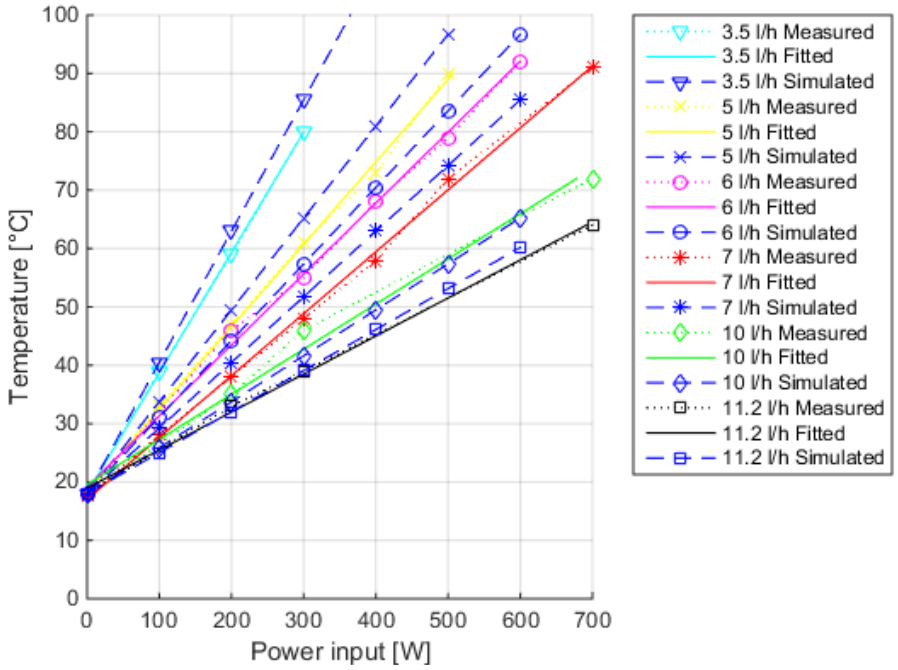


Figure 6.3: Simulated and measured maximum temperatures for different flow rates and input power levels

The gradient of each of the flow rate curves was determined. The linear relationship between these gradients and flow rate, shown in Figure 6.4, can be used to calculate the heating gradient for any flow rate within the microwave system's operating range. This relationship will be used in the control system developed in Chapter 7: Control System Design.

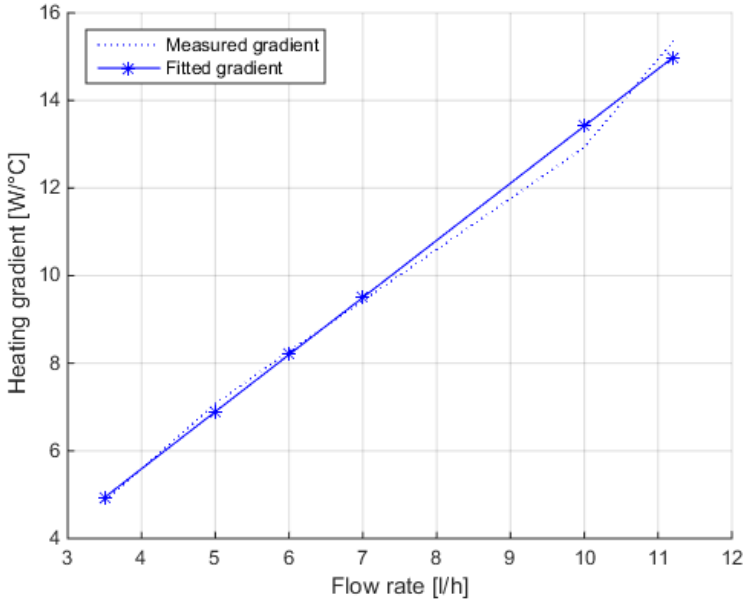


Figure 6.4: Heating gradients for different flow rates

6.4.4 Steam Chamber Calculations

Steam chambers at 100 °C and 120 °C were simulated to compare microwave heating with convective heating. These temperatures were calculated using eq. 6-14 and the comparison is shown in Figure 6.5. The microwave system does not heat above 100 °C as there is no pressure in the system to allow temperatures above boiling point.

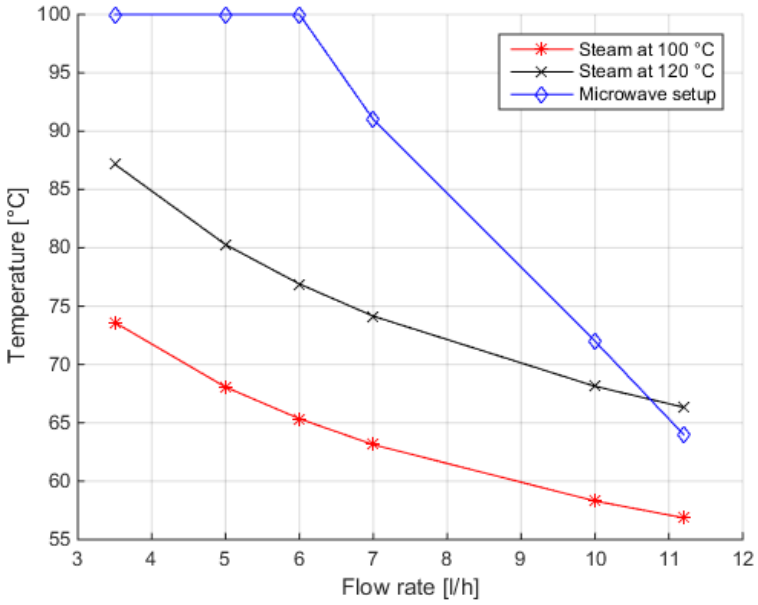


Figure 6.5: Comparison of maximum temperatures reached by the microwave setup and the steam chambers

6.5 Chapter Closing

This chapter covered the heat transfer for the microwave system. It was shown that the load will absorb all the power in the cavity and therefore the magnetron will not be damaged by the reflected power. A heating gradient was determined that is dependent on the flow rate used in the microwave system. The microwave system was also compared to steam chambers and it was shown that the microwave system can reach much higher temperatures within the same time. This will reduce the heat exposure time for the growth media in the final system, which will help preserve vital nutrients. The next chapter develops the PI controller for the final system.

Chapter 7:

Control System Design

7.1 Chapter Summary

The control system is part of the software developed for the final project as shown in Figure 7.1.

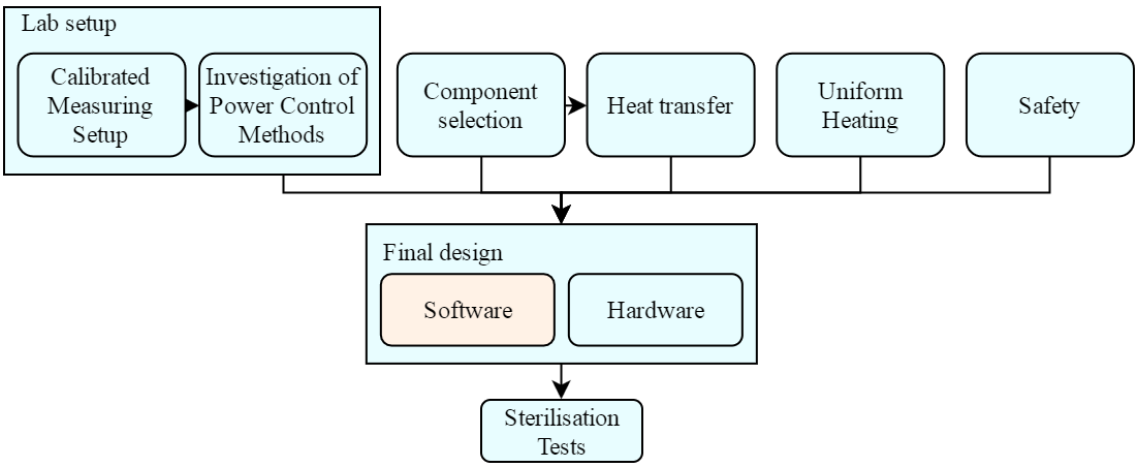


Figure 7.1: The control system within the project development flow

The control system developed in this chapter has to monitor the exit temperature of the growth media and adjust the microwave power level to achieve the temperature set point. The theoretical development of the controller is discussed and then the calculations of the PI-controller are done. Lastly, the controller is adjusted to compensate for the initial empty coil on start-up.

7.2 Theory

The control system for this project uses the exit temperature as feedback to the controller. The Matlab controller then uses the error signal to send the new triac delay value to the triac controller circuit which adjusts the microwave power. The feedback control block diagram is shown in Figure 7.2.

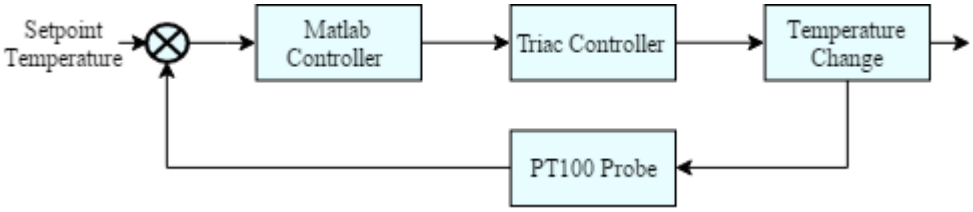


Figure 7.2: Feedback control diagram

The Ziegler-Nichols tuning method was selected for the design of the controller. This method determines the PID control parameters based on the process reaction curve or step response of the system, as illustrated in Figure 7.3.

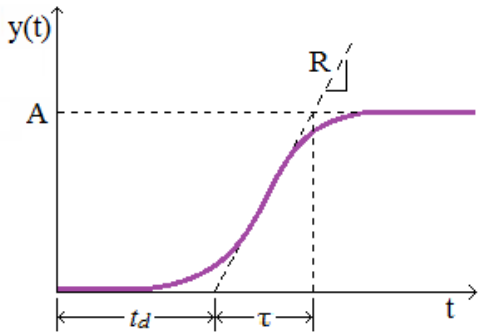


Figure 7.3: Process reaction curve [32]

Based on this S-shape step response, the system can be approximated as a first order system with a time delay, t_d, and a plant gain, A. The transfer function of such a system is:

$$\frac{\boldsymbol{Y(s)}}{\boldsymbol{U(s)}} = \frac{\boldsymbol{Ae^{-st_d}}}{\boldsymbol{s}} \qquad \textbf{7-1}$$

The reaction rate, R, is determined by the tangent at the inflection point of the S-curve:

$$R = \frac{A}{\tau} \tag{7-2}$$

The Ziegler-Nichols step response method results in a quarter decay ratio for the closed loop step response which corresponds to a damping ratio of $\zeta = 0.21$. This may not be ideal for many applications, but it does provide good stability and quick response.

For a controller defined as:

$$D_C(s) = k_p\left(1 + \frac{1}{T_I s} + T_D s\right) \tag{7-3}$$

The control parameters for different types of controllers are defined in Table 7-1.

Table 7.1: PID control parameters as defined by the Ziegler-Nichols method

Type of controller	**Parameters**
P	$k_p = 1/Rt_d$
PI	$k_p = 0.9/Rt_d$
	$T_I = t_d/0.3$
PID	$k_p = 1.2/Rt_d$
	$T_I = 2t_d$
	$T_D = 0.5t_d$

The analog controller has to be converted from the s-plane to the discrete z-plane to find the digital control system. Using the trapezoid rule, also known as Tustin's Method, the discrete equivalent to $D_a(s)$ is [32]:

$$D_d(z) = D_a\left(\frac{2}{T_s}\frac{z-1}{z+1}\right) \tag{7-4}$$

From this, the complete discrete PID controller can be found with a sample time of T_s as:

$$D_C(z) = k_p\left(1 + \frac{1}{T_I}\frac{T_s}{2}\frac{z+1}{z-1} + T_D\frac{2}{T_s}\frac{z-1}{z+1}\right)$$

7-5

This discrete controller needs to be transformed to the difference equation that will calculate the next microwave power control output signal by taking into account the current control signal as well as the current and previous errors between the set point and measured temperatures.

This can be done by getting $D_c(z)$ into the following standard form:

$$D_c(z) = \frac{E_1 z - E_2}{U_1 z - U_2}$$

7-6

The constants E_1, E_2, U_1 and U_2 will be determined by the controller parameters. The difference equation to be implemented in the PI control program can be found as:

$$u(k+1) = \frac{U_2}{U_1}u(k) + \frac{E_1}{U_1}e(k+1) - \frac{E_2}{U_1}e(k)$$

7-7

Where time step $k+1$ is the current values and k is the previous values.

The parallel form of the PID controller was also used to gain a better understanding of the relationship between the controller parameters and the final implemented digital difference equation [33]:

$$D_C(s) = K_p + \frac{K_i}{s} + K_d s$$

7-8

With: $K_p = k_p$

$K_i = k_p/T_i$

$K_d = k_p T_d$

The discreet controller can be found as [33]:

$$D_c(z) = \frac{K_1 + K_2 z^{-1} + K_3 z^{-2}}{1 - z^{-1}}$$

7-9

Where: $K_1 = K_p + K_i + K_d$

$K_2 = -K_p - 2K_d$

$K_3 = K_d$

This lead to the final PI difference controller [33]:

$$u(k+1) = u(k) + K_1 e(k+1) - K_2 e(k) \quad \textbf{7-10}$$

Using this form, the final controller values can be adjusted without the need to redo the z-transform with each change. The PID controller values affect the controller as shown in Table 7.2.

Table 7.2: Effects of the PID control variables [32]

Variable	**Rise time**	**Overshoot**	**Settling time**	**Error**
K_p	Decrease	Increase	Small increase	Decrease
K_i	Decrease	Increase	Increase	Eliminates
K_d	Small change	Decrease	Decrease	No effect

7.3 Calculations

This process of finding the final difference equation was applied to the current system with a step input of 100 W. The measured temperature curve will be used to develop the continuous PI control of the system before the system is adjusted for start-up conditions.

7.3.1 Continuous PI Controller

The temperature curve obtained from this step change can be seen in Figure 7.4.

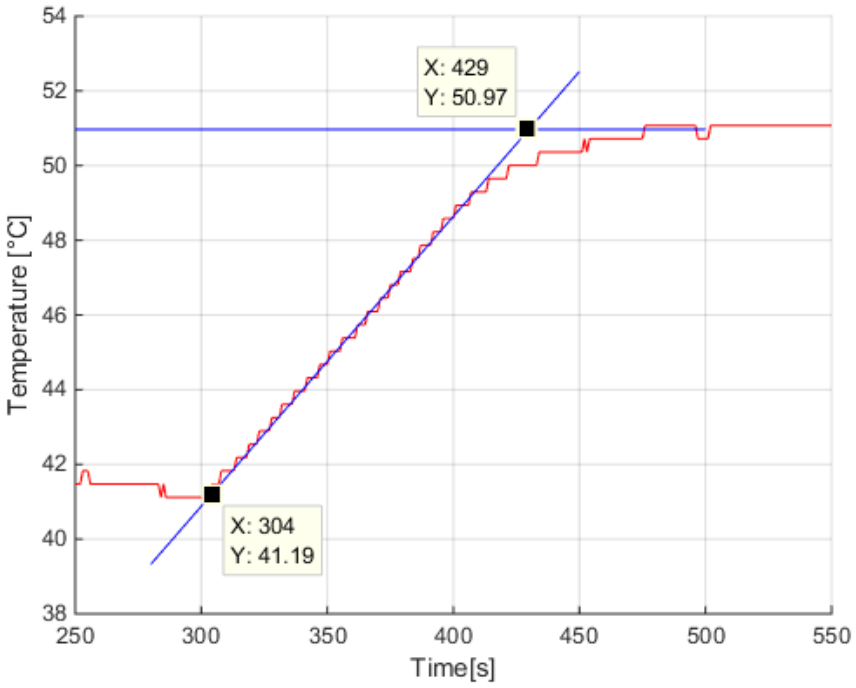


Figure 7.4: Process reaction curve to a 100 W step change

The step change was implemented at time 300 s which means there is a delay time, t_d, of 4 seconds. A sampling period of 1 s was used for the control system measurements. The values of all the parameters discussed previously were calculated using the measured data for a PI controller and can be seen in Table 7-3.

Table 7.3: Calculated control parameter values

Parameter	Value
A	9.78 °C
τ	125 s
$t_d = L$	4 s
R	0.07824 °C/s
k_p	2.876
T_i	13.33

The trapezoidal rule can be implemented by hand or by using Matlab's *c2d* function. This yields the following discrete controller:

$$D_c(z) = \frac{3.017z - 2.799}{z - 1} \qquad \textbf{7-11}$$

Matlab was used to simulate a close loop step response to the designed controller. Figure 7.5 shows the response with the quarter decay ratio visible.

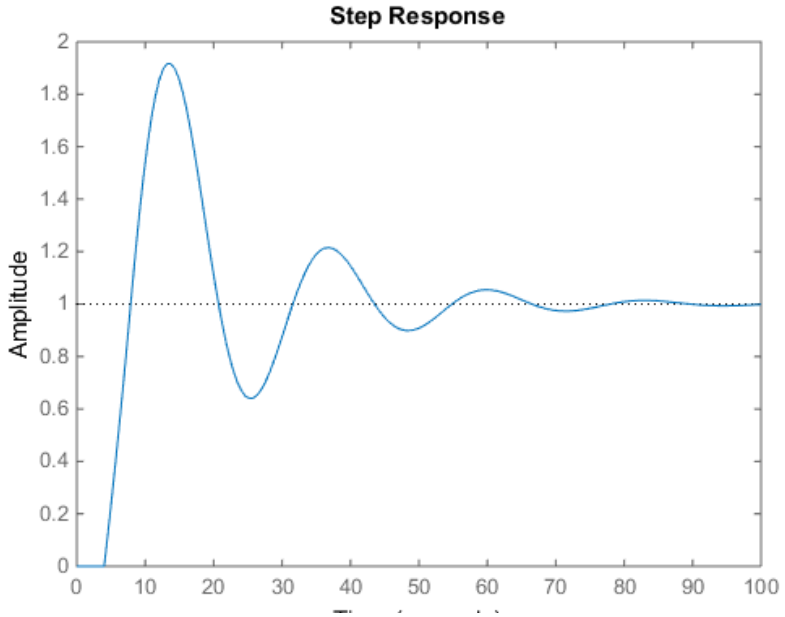


Figure 7.5: Closed loop step response

Because of concerns that such high overshoot may cause boiling and thus pressure build up in the system at higher target temperatures, the K_p and K_I value were adjusted to reduce the oscillation and overshoot. The new closed loop response curve is seen in Figure 7.6.

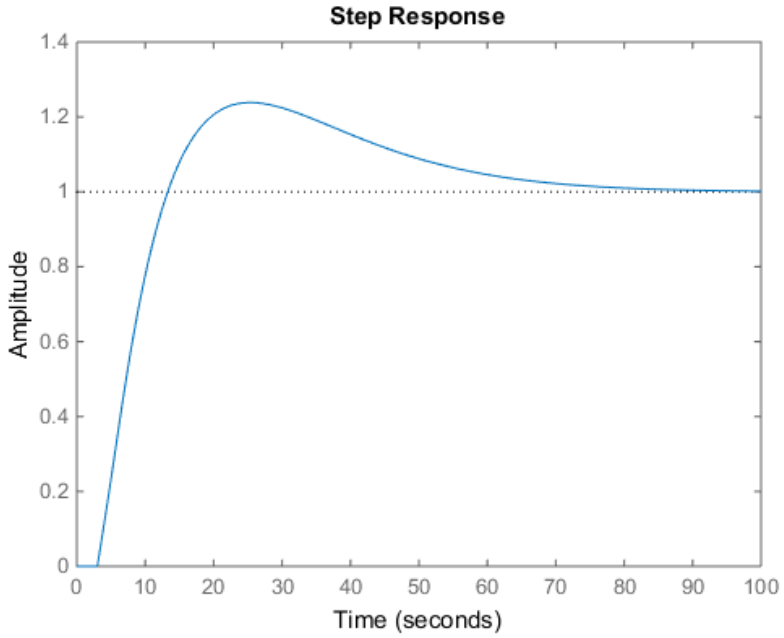


Figure 7.6: Improved closed loop step response

Lastly, the final difference equation to be implemented is:

$$u(k+1) = u(k) + 1.5043e(k+1) - 1.45e(k) \qquad \textbf{7-12}$$

This equation sets the new required power level, *u(k + 1)*, based on the current and previous error, *e(k+1)* and *e(k)* respectively, between the target temperature and the measured exit temperature as well as the current power level, *u(k)*.

7.3.2 Adjustments for Batch Tests

The control system is dependent on the exit temperature of the setup. Because the coil still needs to be filled during start-up of the system, a method was developed to account for this initial lack in accurate exit temperatures. This method uses the heating gradient developed in Chapter 6: Heat Transfer, shown in Figure 6.3.

The volume of the tube, V_{tube} [l], is known as well as the flow rate, $\dot{V}$ [l/s]. Using this, the time it takes to fill the coil, t_{tube} [s] can be calculated as:

$$t_{tube} = \frac{V_{tube}}{\dot{V}} \tag{7-13}$$

The heating gradient, M [W/°C], is given by the equation:

$$M = 1.304\dot{V} + 0.3744 \tag{7-14}$$

The inlet and target temperature is known, therefore, by using the heating gradient the initial power level, P_{init} [W], can be set to assure that the exit temperature will be at, or close to, the target.

$$P_{init} = (T_{target} - T_{in})M \tag{7-15}$$

After the tube is filled, the continuous PI control system takes over to keep the temperature constant and adjust the power if the target temperature is changed.

7.4 Chapter Closing

This chapter developed the control system for the exit temperature of the growth media. The continuous PI controller was designed first and then adjusted to compensate for the empty coil at the initial start-up of the system. The next chapter discusses the final system's hardware and software.

Chapter 8:

Final Design

8.1 Chapter Summary

This chapter discusses the hardware and software of the final system as indicated in Figure 8.1 as part of the main project development.

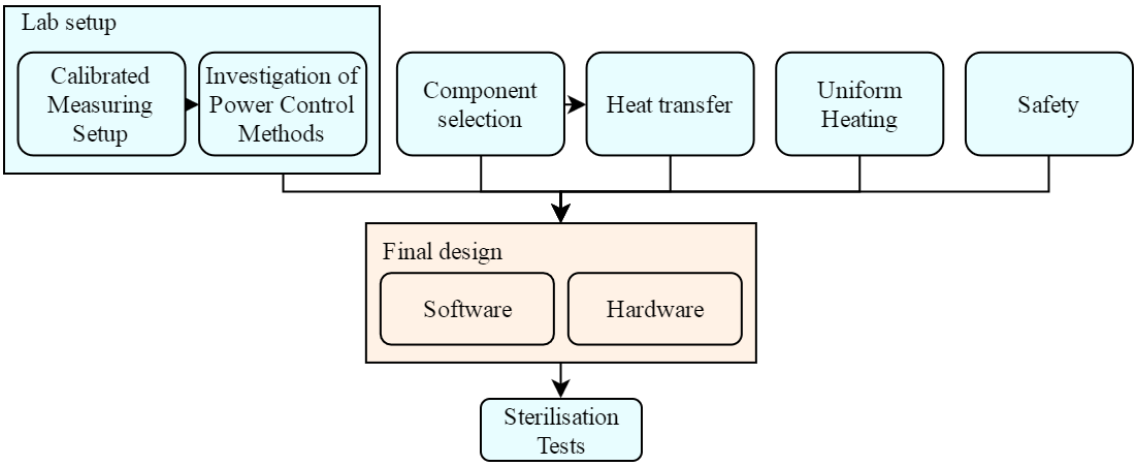


Figure 8.1: The final design as part of the main project.

This chapter will give an overview of the final system assembly before discussing all circuits required with the exception of the microwave power control circuits which were discussed in Chapter 3: Measurement Setup and Microwave Power Control. The software for the main Matlab program as well as both Arduinos used as microprocessors is discussed.

8.2 System Overview

Figure 8.2 shows the complete final microwave setup before sterilisation tests were started. This includes the pump section, the modified domestic microwave oven and all circuits required.

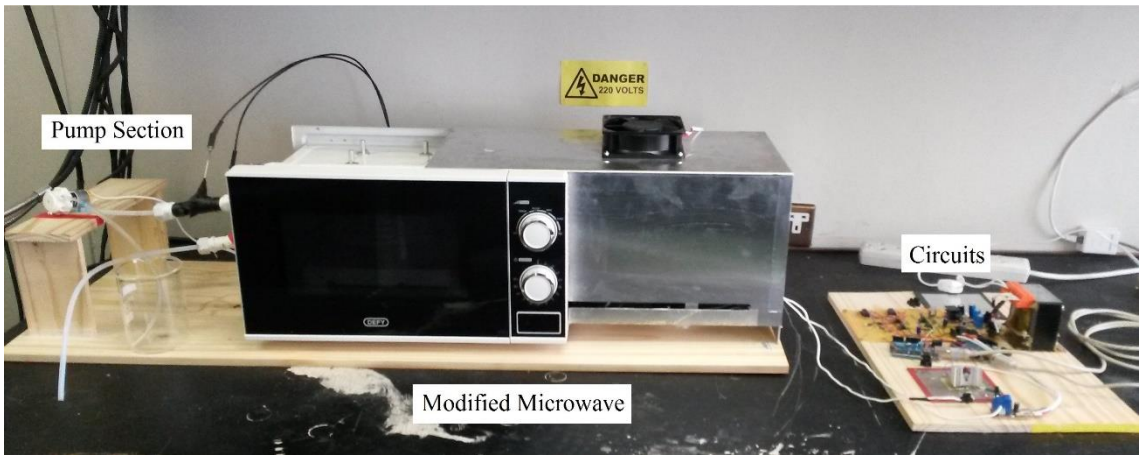


Figure 8.2: Final system assembly

The transformers were connected to allow the anode current to be controlled and were then enclosed in an aluminium housing for safety. The transformers circuit is repeated in Figure 8.3 below.

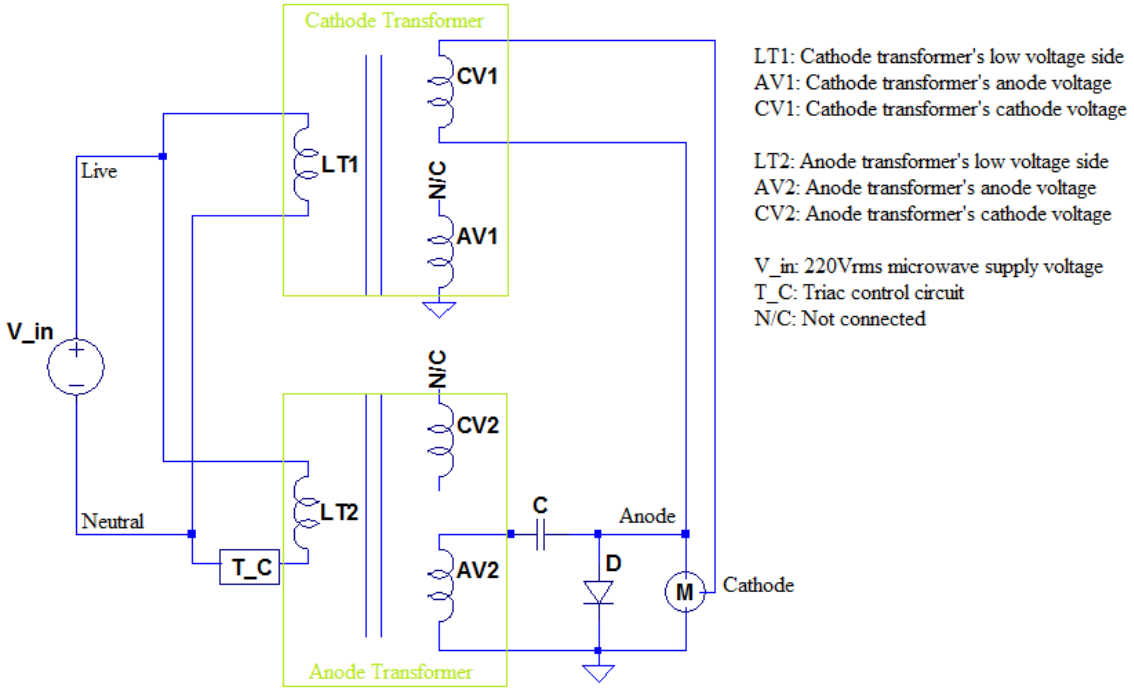


Figure 8.3: Transformer connection to allow anode current control

Figure 8.4 shows the physical transformers connected to the magnetron.

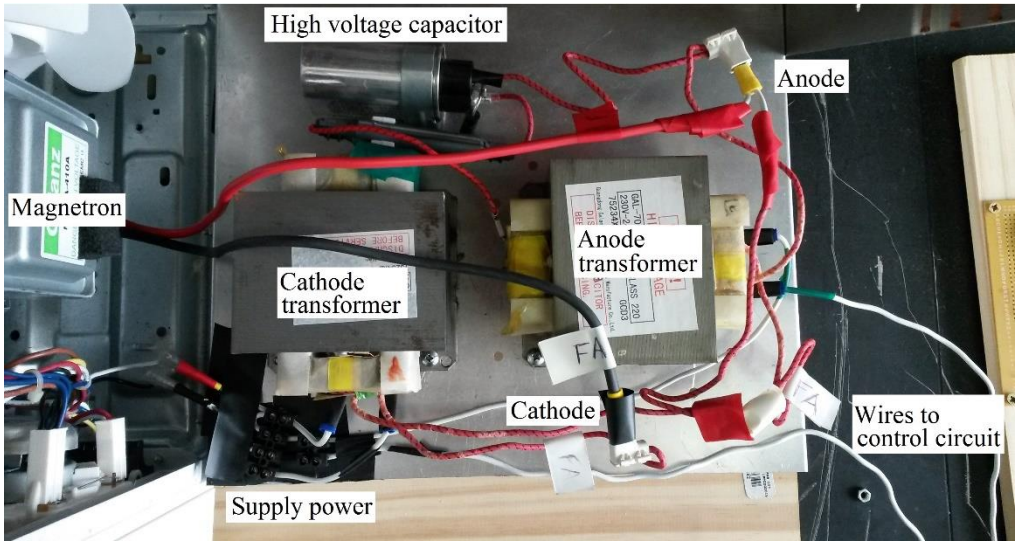


Figure 8.4: High voltage transformers connected to allow anode current control

The remaining hardware will be discussed in the following sections. Figure 8.5 shows the system diagram with the interactions of the different components.

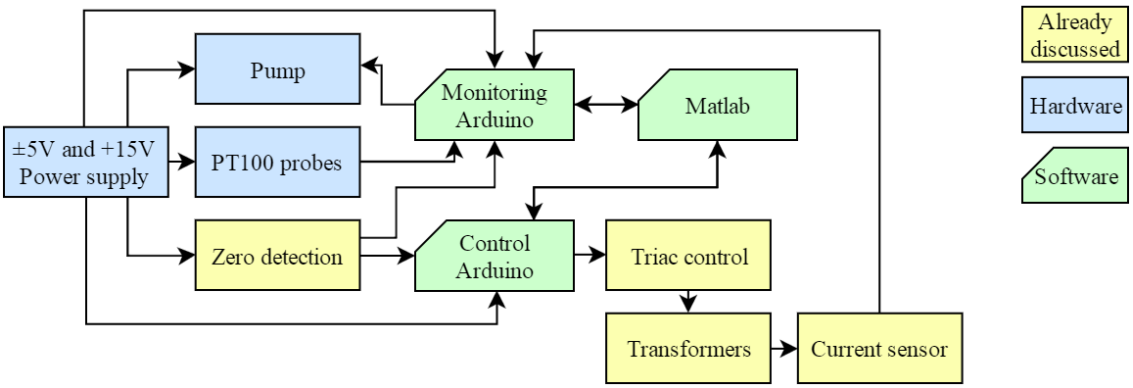


Figure 8.5: Component interactions of the final system

8.3 Hardware

This section will focus on the circuitry for the final system which has not been covered in previous sections. A close-up of the physical circuits is shown in Figure 8.6.

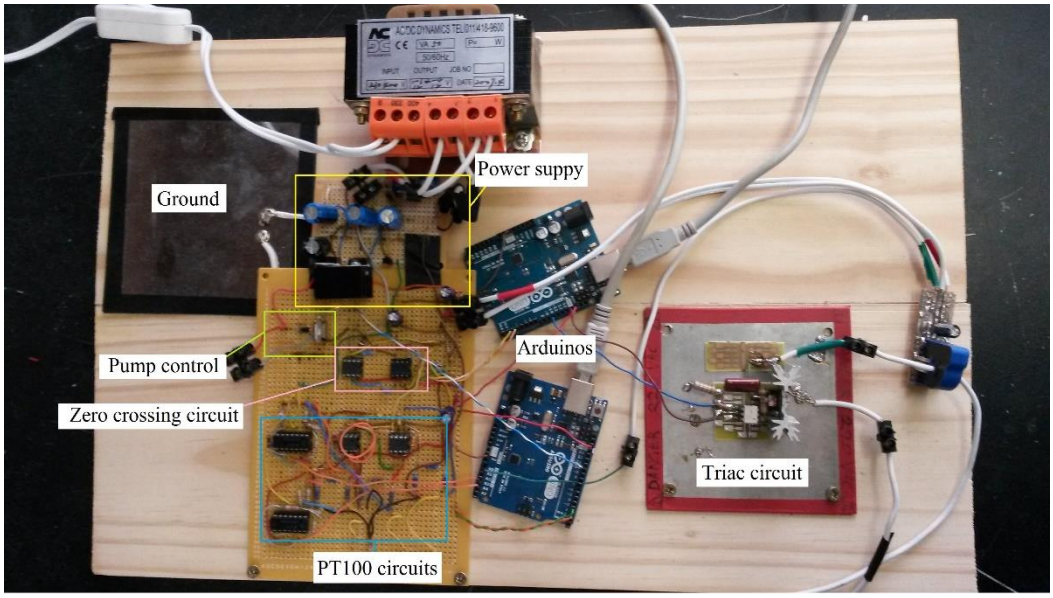


Figure 8.6: Circuits for the final setup

8.3.1 Power Supply

The system uses a ±5 VDC for the circuitry and 15 VDC for the pump. These power supplies were built using linear voltage regulators. LM78L05 and LM7905 voltage rectifier IC's were used to produce the ±5 VDC supply. A L7815CV voltage rectifier IC was used to produce the 15 VDC supply for the pump.

8.3.2 PT100 Temperature Probes

The PT100 probes used for the inlet and exit temperatures each have a measuring circuit as the one shown in Figure 8.7 below.

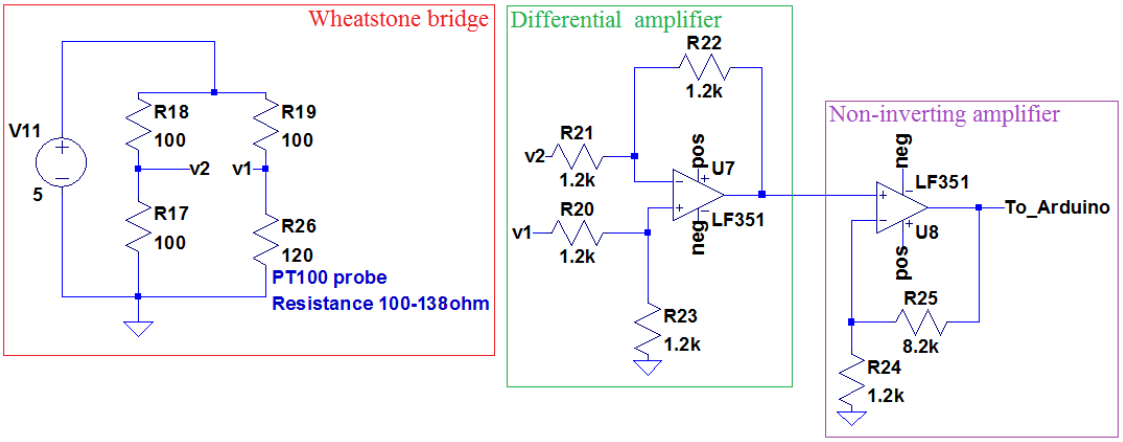


Figure 8.7: PT100 measuring circuit

The probe's wires are connected in a Wheatstone bridge of 100 ohm resistors, this will result in the bridge being balanced at 0 °C. The voltage output of the bridge is connected to a difference amplifier to determine the change in voltage as the temperature changes. Finally the signal is amplified with a non-inverting amplifier and read by the Arduino.

Current flowing through the PT100 probe will cause heat dissipation, as with any resistor. This can lead to self-heating within the probes which can cause errors in measured temperature. PT100 probes have specified dissipation constants based on their construction and the medium in which they are used. The self-heating of PT100 probes in free air can be up to 100 times greater than in water. The dissipation constants are given as mW/°C and indicates the dissipated power required to cause 1 °C error due to self-heating [34].

The probes used in this project have a dissipation constant of 65 mW/°C in water [35]. The most power will be dissipated when the PT100 probe is at its lowest resistance value, 100 Ω. The power dissipated in this case can be calculated to be 62.5 mW. This leads to a 0.96 °C error in the temperature measurements for the worst case scenario. This shows the PT100 temperature measurements are within the project specifications.

8.3.3 Peristaltic Pump

The 15 VDC pump's flow rate is driven by a PWM signal from the monitoring Arduino to the pump motor. The driver circuit can be seen in Figure 8.8.

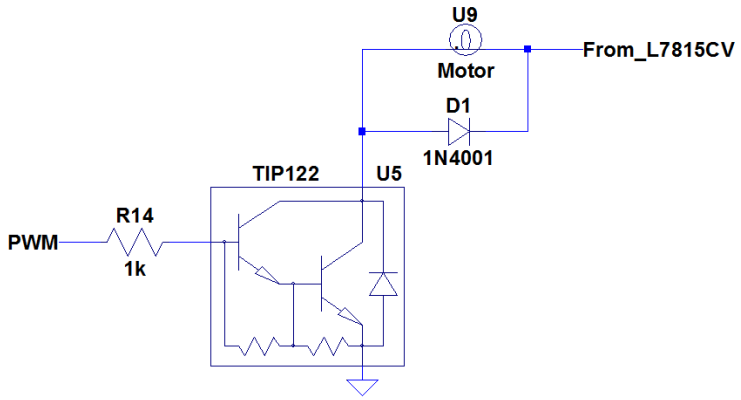


Figure 8.8: DC Pump PWM driver circuit

8.4 Software

The control of the system is implemented using two Arduinos as microprocessors. For this project, the Arduino's speed and resolution is sufficient and the control algorithms can be easily implemented. The one Arduino is used to control the magnetron power via triac triggering and the other to read in the temperature probes and the current sensor and to control the pump. Both Arduinos use serial communication to connect with the main control program operating in Matlab.

8.4.1 Matlab Program

Figure 8.9 shows the flow of the main Matlab control file.

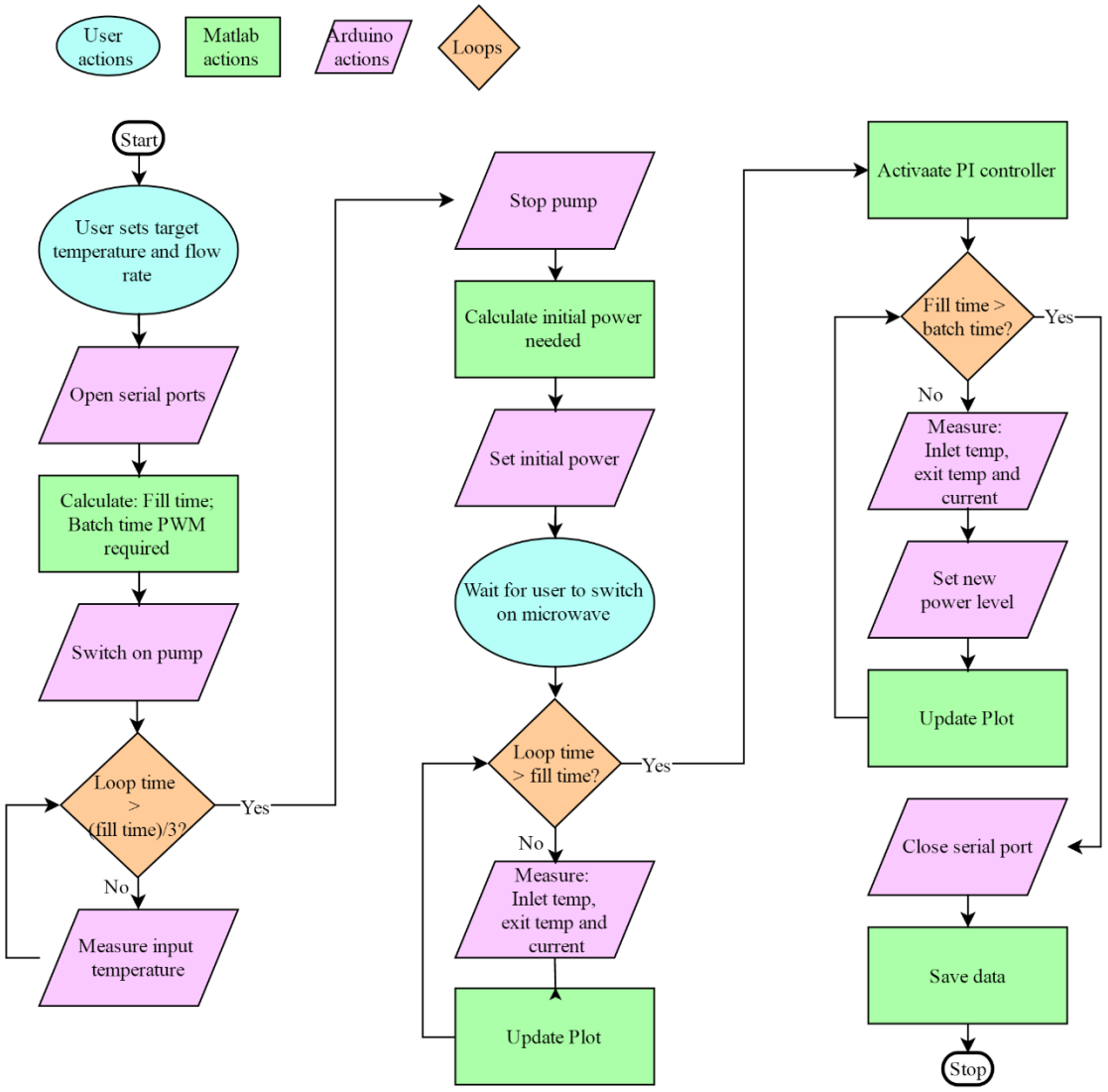


Figure 8.9: Program flow of the main Matlab control program

The control program starts with the user setting the desired exit temperature and the flow rate needed as well as the test batch size. Matlab then establishes serial connections with both of the Arduinos via the specified serial ports. Based on the flow rate and batch size, the time it takes to fill the coil as well as the time it will take to pass the entire batch through the microwave oven is calculated. The PWM value required for the pump to

achieve the desired flow rate is also calculated. This value is then sent to the monitoring Arduino which starts the pump.

The first loop allows the coil to be filled approximately a third of the way. This is done to reduce the initial overshoot of the exit temperature. During this loop the inlet temperature is measured every one second. Matlab sends a command to the monitoring Arduino to request the inlet temperature. The monitoring Arduino records the measurement and sends it back to Matlab which then saves it.

When this loop is complete, the pump is switched off by sending a PWM value of 0 to the monitoring Arduino. Matlab then calculates the initial microwave power required by using the difference between the average measured input temperature and the required exit temperature along with the heating gradient calculated for the specific flow rate.

Using this calculated initial power, the triac delay time is calculated and sent to the power control Arduino. The program is then paused until the user allows it to continue. This is done because the user has to manually switch on the microwave oven as a safety precaution. Once the user confirms the program can continue, the pump is switched back on at the same flow rate by again sending the calculated PWM value to the monitoring Arduino.

The second loop runs until the coil is filled completely. During this loop Matlab sends commands to the monitoring Arduino every one second to request both temperature readings as well as the current reading. The current value is converted to power to confirm that the output power is as expected. These values are plotted on a live updated graph with every new reading so that the user can monitor the process.

Once the coil is filled the exit temperature can be accurately measured and the PI controller is activated. The third and final loop runs until the entire batch has been passed through the microwave. The current and previous exit temperature errors are calculated and used to determine the new required microwave power level. This new required power is used to calculate the new triac delay time which is then sent to the control Arduino. The rest of this loop is the same as loop two: measure the inlet and exit temperatures as well as the current, convert the current to power and update the graph.

When the final loop is complete, the system is switched off by stopping the pump and ending serial communication with both the Arduinos. Finally, all the measured data is saved in .csv files for later use.

8.4.2 Monitoring Arduino Program

The monitoring Arduino runs in an infinite loop in which the readings are timed with an interrupt triggered by the rising edge of the zero crossing. Figure 8.10 shows the flow of the program running on this Arduino.

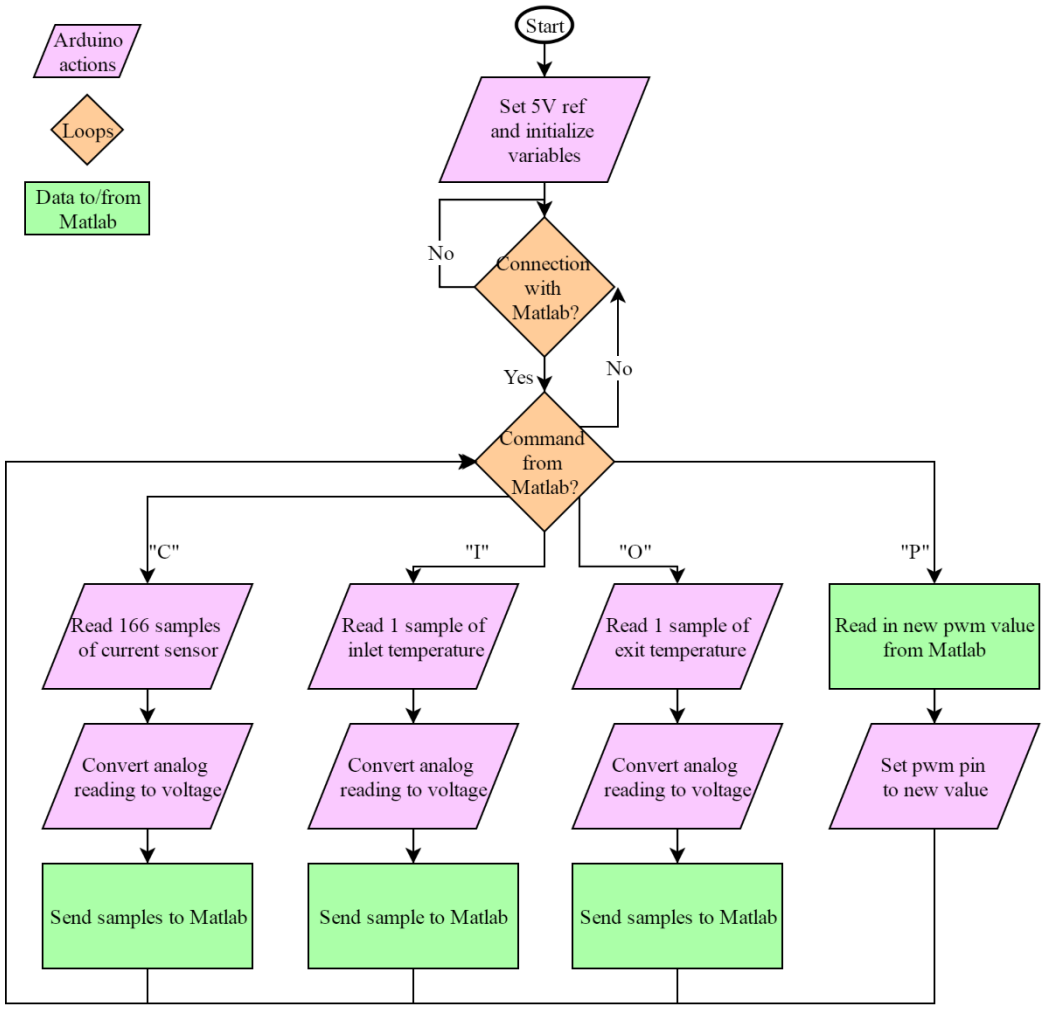


Figure 8.10: Monitoring Arduino program flow

Both Arduinos use an external 5 V signal as a reference instead of the internally generated 5 V. This is to ensure the circuits and the Arduinos have the same reference voltage. This is set using the "*analogReference (EXTERNAL)*" command and reading an analog pin immediately afterwards. This must be done to avoid the Arduino shorting internally and damaging the processor. After this reference is set, the rest of the pins can be initialised safely.

The Arduino enters a loop waiting for Matlab to establish a serial communication. Once the communication is active the Arduino waits for commands from Matlab by constantly monitoring the serial port. This program works with four different commands:

- 'C': Arduino saves 166 (20 ms) samples of analog current measurement data, converts it to relative voltage values and sends it to Matlab for processing.
- 'I': Arduino saves 1 sample of analog inlet temperature data, converts it to a relative voltage value and sends it to Matlab for processing.
- 'O': Arduino saves 1 sample of analog outlet temperature data, converts it to a relative voltage value and sends it to Matlab for processing.
- 'P': Arduino reads in the new PWM value for the pump and sets the PWM pin to this new value.

The analog data is converted to relative voltage values using the following equation:

$$D_{voltage} = \frac{5D_{analog}}{1024} \quad \textbf{8-1}$$

Where $D_{voltage}$ is the relative voltage signal of the analog data value, D_{analog}.

8.4.3 Control Arduino Program

The program flow for the control Arduino is shown in Figure 8.11. This is an infinite loop program that uses both rising and falling edge interrupts triggered by the zero crossings.

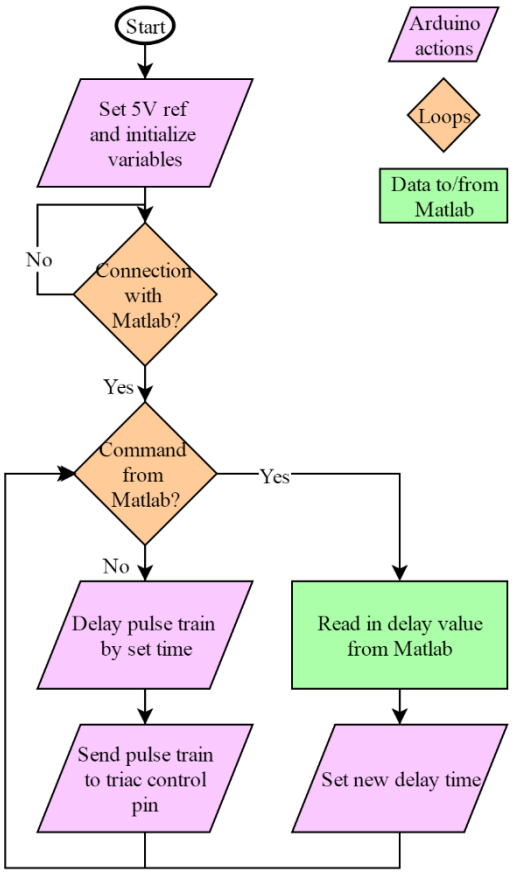


Figure 8.11: Program flow of the control Arduino

Similar to the monitoring Arduino, the control Arduino also first sets the external reference voltage and then waits to establish a serial connection with Matlab. When the Arduino is connected it moves into a loop that constantly transmits a delayed pulse train to the triac control circuit. This pulse train is delayed by the triac delay time to achieve the desired microwave power levels. The rising and falling edge interrupts of the zero crossing each represents half of the period of the supply voltage frequency, 50 Hz. Each half cycle delays the pulse train by the set triac delay time.

The serial port is also monitored in each rising edge interrupt to check for a new triac delay time requested from Matlab. When a new value is available it is read in and the delay time for the triac is changed accordingly.

8.5 Sample System Results

Two example tests to show how the final setup works is discussed here. Using the equations discussed in Chapter 6: Heat Transfer the initial magnetron output power was calculated for the target temperatures. Table 8.1 gives a summary for these two tests.

Table 8.1: Sample test summary

Test	Input temp	Target temp	Flow rate	Heating gradient	Initial power
1	18 °C	50 °C	5 l/h	6.894 W/°C	220.6 W
2	40 °C	90 °C	5 l/h	6.894 W/°C	344.7 W

Figure 8.12 shows the temperature and power results for test 1.

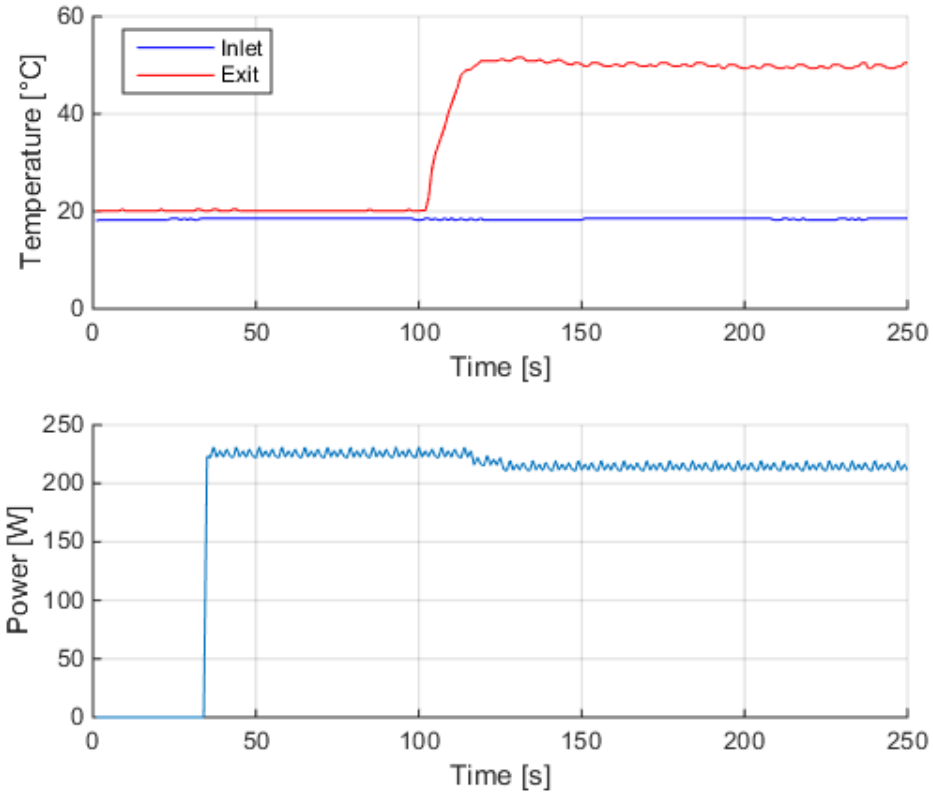


Figure 8.12: Test 1 results

The initial 101 seconds is the time it takes for the coil to fill. During this time the exit temperature sensor only measures the air temperature inside the coil. After 34 seconds the coil is filled a third of its volume and the power is switched on. The power remains constant until the PI controller takes over once the exit temperature can be accurately measured. The controller then keeps the output temperature at the target temperature.

Figure 8.13 shows the results of test 2. This test was conducted with a higher inlet temperature.

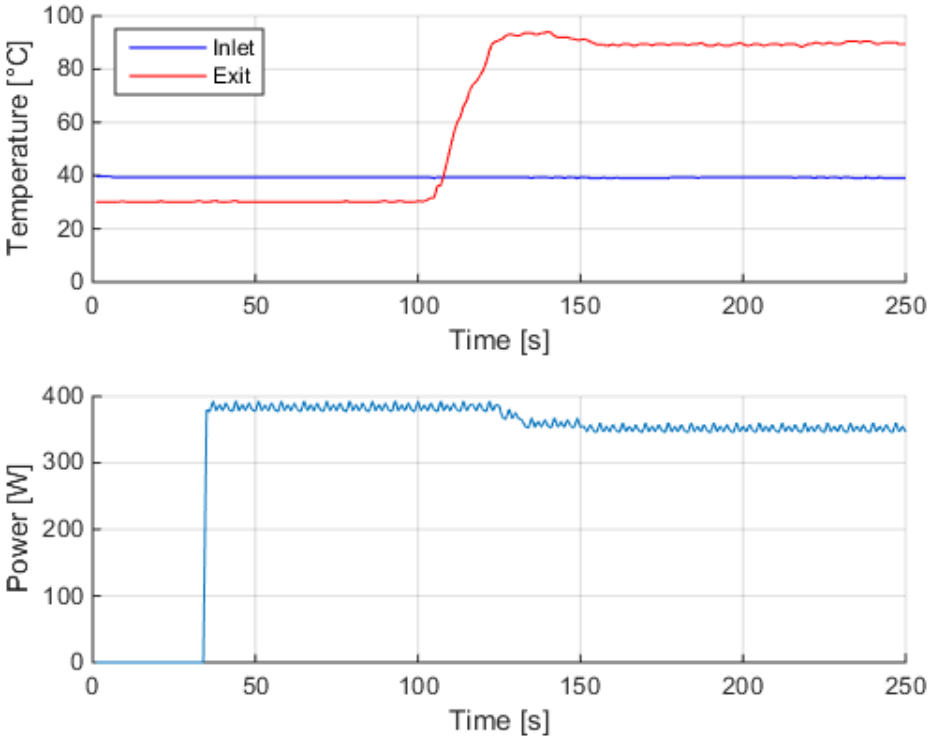


Figure 8.13: Test 2 results

Again, the first 101 seconds only measures the air temperature inside the coil. After the coil is filled a third of its volume the PI controller takes over and reduces the power to achieve the set temperature.

8.6 Chapter Closing

This chapter discussed the final system assembly. The circuits for the hardware were given, except for the power control circuits. The software programs of both Arduinos as well as the main Matlab program were also discussed. Sample test results were given to show the system works.

The next chapter focuses on the sterilisation tests done on contaminated growth media using this final setup.

Chapter 9:

Sterilisation Tests

9.1 Chapter Summary

The final setup was moved to the BIOPEP Peptide Group laboratory in the Biochemistry Department to conduct the sterilisation tests on growth media. These tests form the final part of the project development as indicated in Figure 9.1.

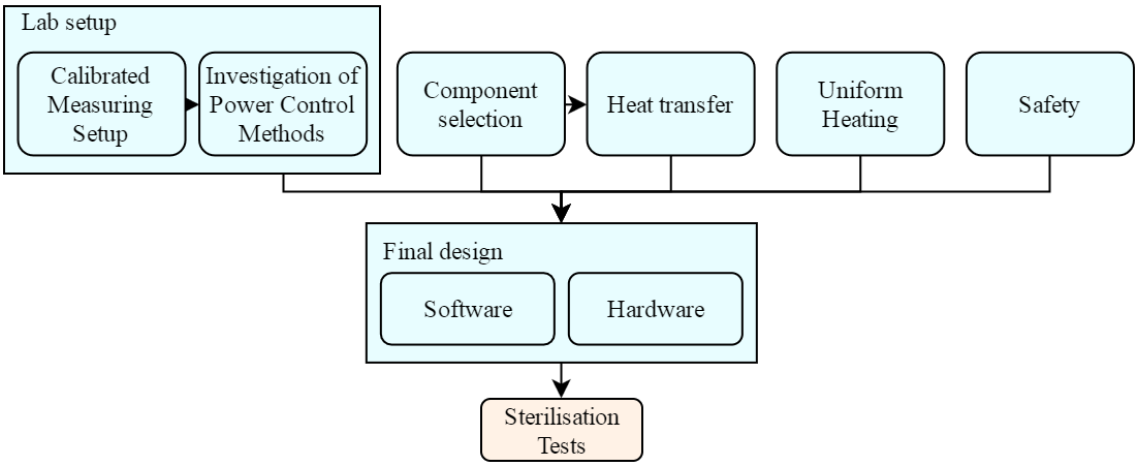


Figure 9.1: Sterilisation tests in the overall project development

The goal of these tests is to prove that a continuous flow system using only microwaves as a source of heat is a valid method of sterilising growth media.

This chapter will discuss theory relating to the tests as well as the pre-test preparations. The test procedure is described followed by a discussion of the different tests along with each result. Finally, the developed microwave system method is compared to the autoclave in terms of energy and time.

9.2 Microorganism Theory

While working with microorganisms, it is important to understand their growth curve. Microorganisms growing in a closed system or batch culture usually exhibits the growth characteristics show in Figure 9.2.

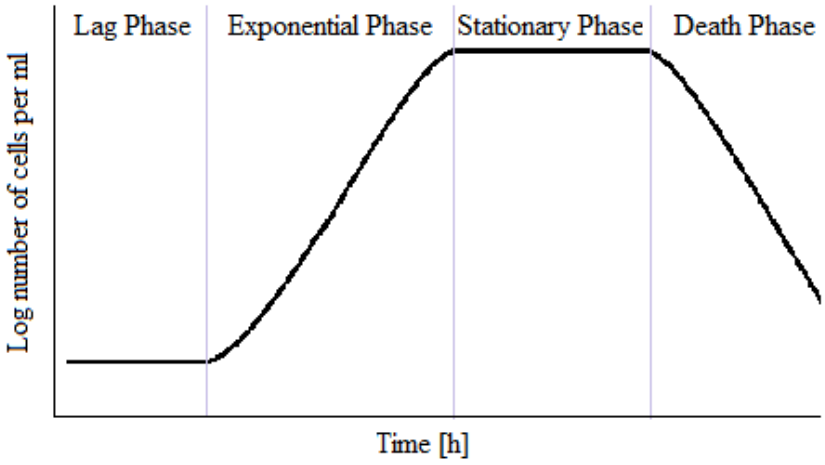


Figure 9.2: General growth curve of microorganisms [36]

Lag phase: A new culture is started by placing a small amount of microorganisms into a batch of sterile media. The organisms do not reproduce immediately because they need to recover from the shock of being transferred to new media and acclimatise to their new environment before they start multiplying [36].

Exponential phase: When the cells are acclimatised, they begin their metabolic process where the cells start dividing by binary fission at a constant rate. The rate depends on the organism and the composition of the media being used. This rate is known as the generation time, which is the time required for the number of cells to double which leads to exponential growth of the cell concentration [36].

Stationary phase: The exponential growth of the organisms cannot be sustained indefinitely due to the exhaustion of nutrients, the limited space and the accumulation of end products which leads to the stationary phase. During this phase it cannot be determined whether cells are dying and reproducing at the same rate or if no new growth is taking place [36].

Death phase: If the culture is left to incubate, the microorganisms enter the death phase. The cells die off due to lack of nutrients at the same rate as the generation time [36].

In the growth curve the concentration of cells is expressed as a log number. It has been shown that this log number of cells can be directly related to the optical density, OD, of the culture which makes it easier to determine the concentration of within a culture. The OD is measured in a spectrophotometer using samples of the culture in specific holders called cuvettes. The spectrophotometer passes light through the sample and measures the light intensity that reaches the photoelectric cell on the other side of the cuvette. Light passes through the cuvette and gets scattered by the cells so not all the light reaches the photoelectric cell, causing a weaker electrical signal. Because sterile media will naturally scatter some light, all measurements are taken in reference to a sample of sterile media. The measurements are also taken with a specific wavelength of the light. It should be noted that this proportionality between OD and cell count is only valid for OD < 0.4 [37].

Different basic organisms should be tested for in order to prove that sterilisation is successful. The most commonly found contaminants are bacteria and yeast. Bacteria is categorised into gram positive and gram negative based on the structural differences in their cell walls using a process known as Gram staining [38]. Gram stain is an alkaline solution of gentian violet or crystal violet. If the bacteria cell retains the colour of the stain it is called Gram positive and if it loses the colour it is classified as Gram negative [39]. Differences in the cell structure such as the thickness of the peptidoglycan layer and the presence of an outer membrane can cause the bacteria to react differently to external stimuli. It is therefore important to test both cases. The basic cell structures can be seen in Figure 9.3.

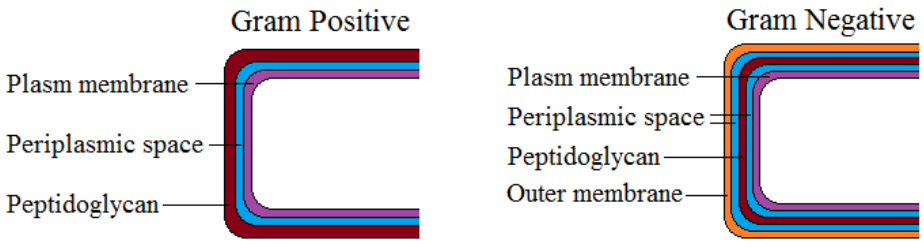


Figure 9.3: Basic representation of bacteria cell wall structure [39]

Yeast is part of the fungi kingdom and, like bacteria, it is a single-celled microorganism. However, yeast has a nucleus containing its DNA, which makes it a complex cell, whereas bacteria is a simple cell [40]. Because the yeast cell structure is more complex, yeast can be more difficult to kill.

Each of the organisms selected grows best in a specific culture media based on their nutrient requirements. This also gives the opportunity to see how different types of media react in a microwave. The following is a brief description of each microorganism selected for tests:

Gram positive: *Micrococcus Luteus* is commonly found on all surfaces, in dirt and water and on human skin. It is a generally harmless bacteria, but can cause infections in people with immune deficiency [41]. This bacteria grows in Trypticase Soy Broth (TSB). The strain used is: NCTC 8340.

Gram negative: *Escherichia Coli* naturally lives in the digestive track of humans and animals. It can also be found in contaminated water and raw foods, especially raw meat. Ingestion of this bacteria can cause severe food poisoning [42]. *E. Coli* grows in Lysogeny Broth (LB). The strain used is: DH5α containing a pGKCherry plasmid.

Yeast: *Saccharomyces Cerevisiae* is commonly known as brewer's yeast. This yeast is used in fermentation to convert sugars into alcohol and it is also used in baking as a rising agent. It is harmless to healthy humans, but can cause fungemia in people with immune deficiency. Fungemia is the presence of yeast of fungi in the blood and presents with flu-like symptoms [43]. *S. Cerevisiae* grows in Yeast Extract-Peptone-Dextrose (YPD).

9.3 Test Preparations

Figure 9.4 sums up all the elements that have to be prepared for each round of tests.

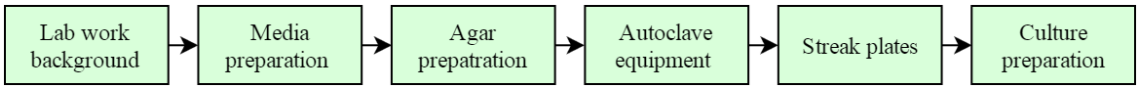


Figure 9.4: Preparation to be done before testing starts

First, some background information about working in the laboratory is explained. The preparations of the media and agar needed is discussed as well as a list of equipment that should be sterilised in the autoclave before use. The preparation of the streak plates is explained as well as the culturing of microorganisms.

9.3.1 Lab Work Background

Laminar flow cabinet:

Figure 9.5 shows a diagram of the airflow within a laminar cabinet.

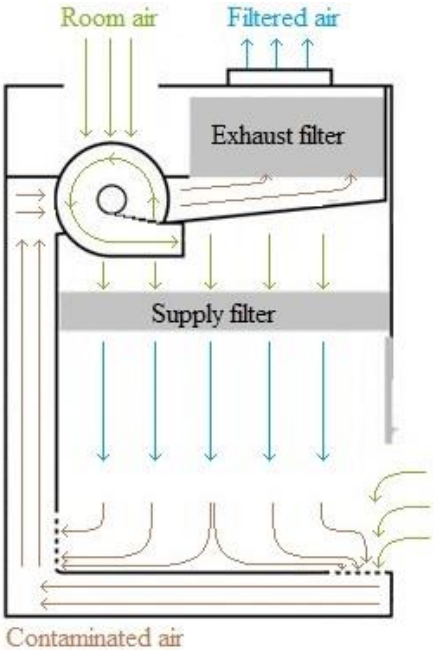
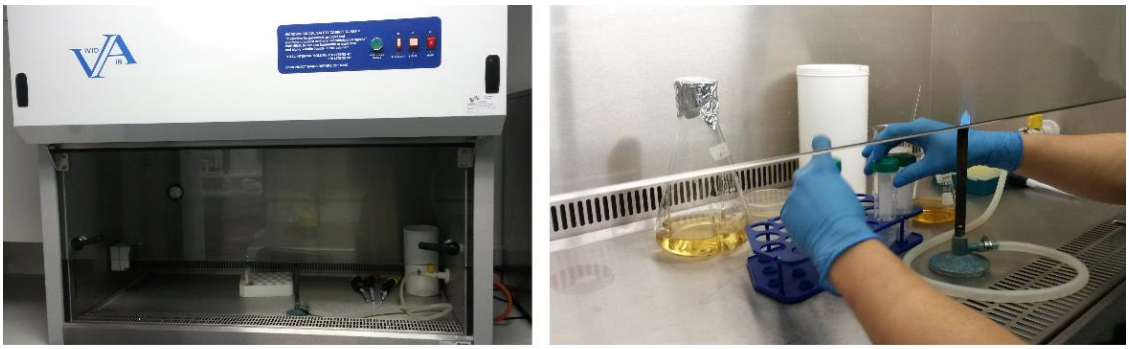


Figure 9.5: Airflow inside a laminar flow cabinet [45]

The laminar flow cabinet provides a sterile work environment. The cabinet is sterilised before every use with UV light. The laminar flow in the front of the cabinet ensures that no contaminants can enter it while in use. The airflow into the cabinet is filtered so no contaminants are introduced into the working space. Exhaust air is also filtered so no potentially harmful contaminates are released into the room air.

All equipment to be used inside the cabinet is sprayed with 70% ethanol before being placed inside. The user wears gloves and also sprays their hands and arms before working inside the cabinet. There is a Bunsen burner available inside the work area if needed and it is good practice to work behind a flame and to use flaming on containers being opened inside the chamber. Figure 9.6 shows the chamber being used during tests.



(a) (b)

Figure 9.6: (a) The laminar flow cabinet; (b) Close-up of tests being done in the cabinet

Ultrapure water:

Normal tap water contains many substances such as sodium and magnesium or dissolved gasses like oxygen or nitrogen that may react unpredictably when used in tests. This can cause the results to be unreliable [44]. For this reason, labs usually use some form of purified water. The Biochemistry Department uses water treated by reverse osmosis for general use, but the water used in the lab for experiments is ultrapure (Type 1) water also referred to as MilliQ water. This is water that has been purified through reverse osmosis and then deionised as well. This is the highest quality of water with virtually no contaminants, thus providing trustworthy results when used in tests [44].

9.3.2 Media Preparation

Preparing TSB media:

The media is made up by adding 30 g of TSB powder to MilliQ water for every 100 ml media required. The media solution is mixed until all the powder is dissolved and then separated into the required containers. Figure 9.7 shows the process of preparing the media. This media should then be sealed and autoclaved to be sterilised.

(a) (b) (c) (d)

Figure 9.7: (a) Weighing out the broth powder; (b) Adding ultrapure water to the powder; (c) Stirring until all the powder is dissolved; (d) Unsterilized media in the correct container

Preparing LB media:

In a similar manner this media used for E.coli is made up by mixing 10 g Tryptone powder, 5 g yeast extract and 10 g Sodium Chloride powder per litre media required. This is then dissolved in MilliQ water, sealed and autoclaved in the same manner as TSB media.

Preparing YPD media:

This media used for yeast has a more complex preparation. First 10 g yeast extract is mixed with 20 g Peptone and dissolved into 900 ml of MilliQ water. This mix is then sealed and autoclaved. Once the media is sterile, 100 ml of sterile 20% glucose is added yielding 1 litre of sterile YPD media. The 20% glucose is made up by dissolving 40 g of

D-glucose in 200 ml MilliQ water and filtering it with a 0.22 µm syringe filter into a sterile container.

9.3.3 Preparing Agar for Streak Plates:

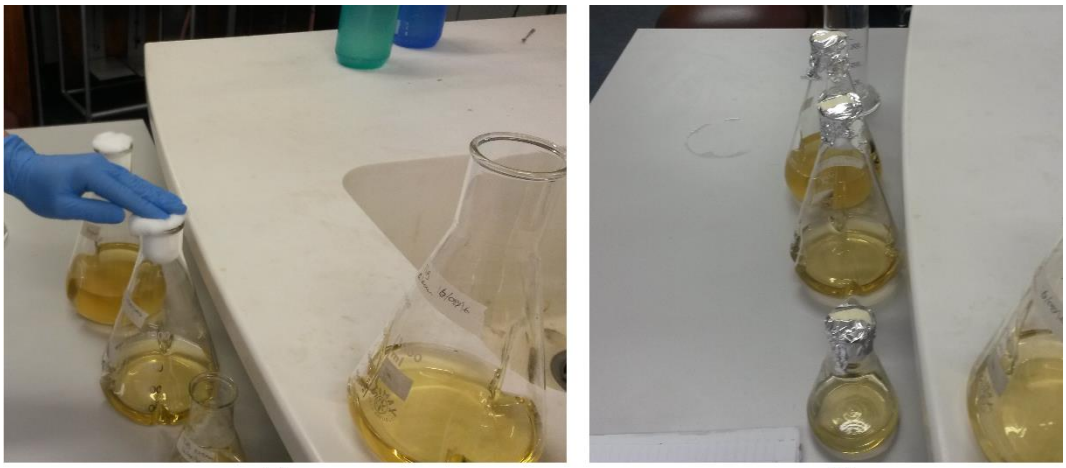
Agar bacteriological powder is added to growth media as a gelatinous thickener. This is used to prepare the streak plates that are used to grow the bacteria colonies on. The powder is added to pre-mixed, but not yet sterilised, media. Agar requires 7.5 g of powder for every 500 ml of media needed. Figure 9.8 shows the agar preparation.

(a) (b)

Figure 9.8: (a) Agar powder measured out; (b) Undissolved agar mixed with media (left) and unsterilized media (right)

Heat is required to dissolve the powder into the media. This heat is provided by the autoclave while the solution is being sterilised. The agar mixture must be used while it is still warm after sterilisation as it will set at room temperature.

If the containers for the media or Agar do not have lids, they are sealed with cotton wool and covered with foil as shown in Figure 9.9. All of these containers are then sterilised in an autoclave.



(a) (b)

Figure 9.9: (a) Sealing flasks with cotton wool; (b) Covering flasks with foil

9.3.4 Equipment and Liquids to be Autoclaved:

For each round of tests the following needs to be sterilised in the autoclave before testing:

- The media required, mix and separated into the correct containers.
- Agar mixture if new streak plates are required.
- Additional containers that are needed to collect the media in during tests.
- Pipette nozzles, petri dishes and test tubes, as needed.
- MilliQ water in sealed containers.

9.3.5 Preparing Streak Plates:

Working in a sterile laminar flow cabinet, the sterile petri dishes are filled with approximately 20 ml of the warm agar and are left to cool and set. The dishes are then covered with their lids and are then ready to be used. This process is shown in Figure 9.10. Note the darker shade of gold of the media due to autoclaving.

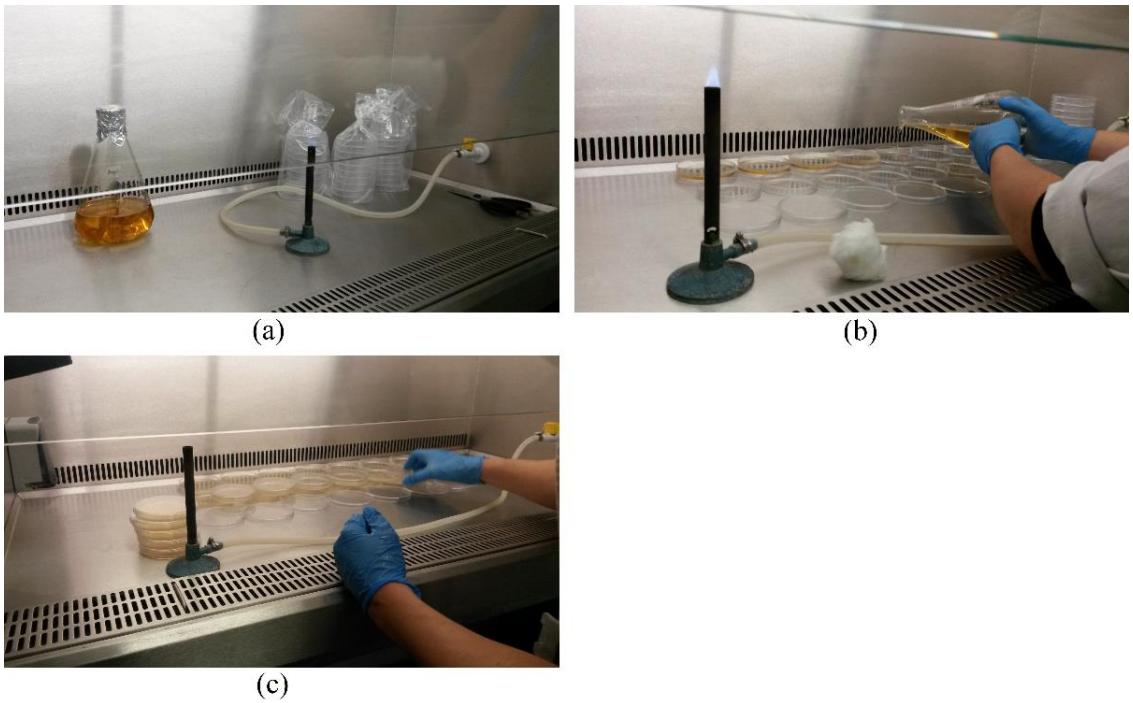


Figure 9.10: (a) Sterile petri dishes and the autoclaved agar mixture in the laminar flow cabinet; (b) Pouring agar into the plates and letting them cool; (c) Covering and stacking the set agar plates

9.3.6 Preparation of Bacterial Culture:

It is important to get the microorganisms in the exponential growth phase of their growth curve for use in tests. This is done by creating a culture of the organism which starts with first streaking out the microorganisms cultures from freezer stocks on the respective media streak plates. These streak plates are then incubated at 37 ˚C for 48 hours, after which 20 ml of media is inoculated with a bacterial colony and incubated overnight on a shaker.

For all tests done the optical density, OD, measurements were taken using a wavelength of 620 nm. The OD_{620nm} of the culture is recorded the following day and 0.5 ml of the

culture is subcultured into fresh media. The subculture is incubated to an OD_{620nm} of ~0.6 and is then diluted to an OD_{620nm} of 0.2 with fresh sterile media before being inoculated into the final testing media batch.

The subculture is diluted by first taking a sample of clean sterile media as well as an undiluted sample of the subculture and placing these in the spectrophotometer to determine the current OD_{620nm}. A small sample of the subculture is placed into a sterile container and a small amount of sterile media is added to this to dilute the concentration. Another OD_{620nm} measurement is taken and this process is repeated until the OD_{620nm} reading is 0.2. If at any point the mixture is too diluted, meaning the reading is below 0.2, a small amount of the original subculture is added to increase the concentration again.

When the OD_{620nm} reading is 0.2 the cell concentration is known. This concentration is then used in the following equation to determine the inoculation volume to be added to the 250 ml media test batch to achieve the desired concentration of the test batch.

$$V_1 C_1 = V_2 C_2 \quad \textbf{9-1}$$

V_1 and C_1 is the inoculation volume to be added and the known cell concentration. V_2 and C_2 the test batch volume and desired concentration respectively.

9.4 Test Procedure

The main sterilisation tests are done using streak plates which allow any surviving microorganisms to grow colonies which can be counted. For the higher microorganism concentration tests, AlamarBlue tests were done as well. These tests measure the metabolism of any remaining organisms and gives an indication of the percentage growth.

9.4.1 Streak Plate Tests

All tests were done in duplicate, meaning two batches of 250 ml were tested for each concentration and microorganism type. Each batch was sent through the microwave twice in case the first pass did not completely sterilise the media. The test procedure discussed in this section is shown in Figure 9.11.

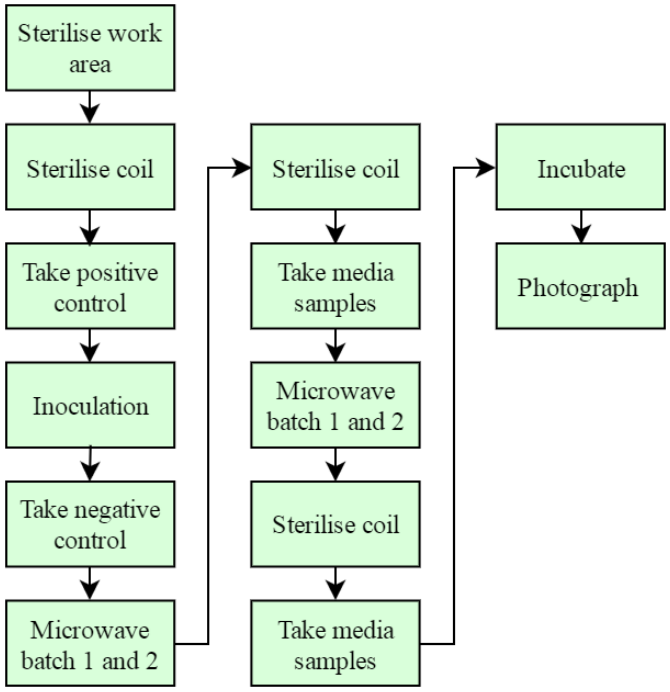
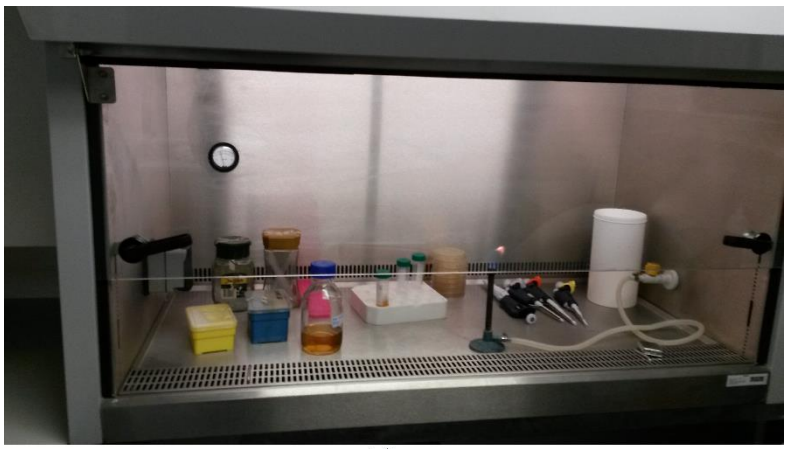


Figure 9.11: Test procedure for two batches passed through the microwave setup twice

The first step is to ensure the laminar flow chamber and the area around the microwave setup is sterile. All work with the media or the streak plates is done inside the laminar flow cabinet. The only time the media is used outside the cabinet is when it is being pumped through the microwave setup.

Figure 9.12 shows the laminar flow cabinet setup for tests as well as the microwave setup area.



(a)

(b)

Figure 9.12: (a) The setup in the laminar flow cabinet with all the tools needed to complete the tests; (b) The microwave setup at a workbench area

At the microwave setup the area surrounding the inlet and outlet of the coil is sprayed with 70% ethanol to sterilise the surfaces. The inside of the coil is also sterilised by first flushing the coil with 70% ethanol and covering both ends with foil as this helps to stop any contaminants falling onto the pipes or into the containers that are being used. The ends of the coil are also flamed each time the containers are changed. The system is then flushed with the sterile MilliQ water to remove any ethanol that may still be in the coil as shown in Figure 9.13.

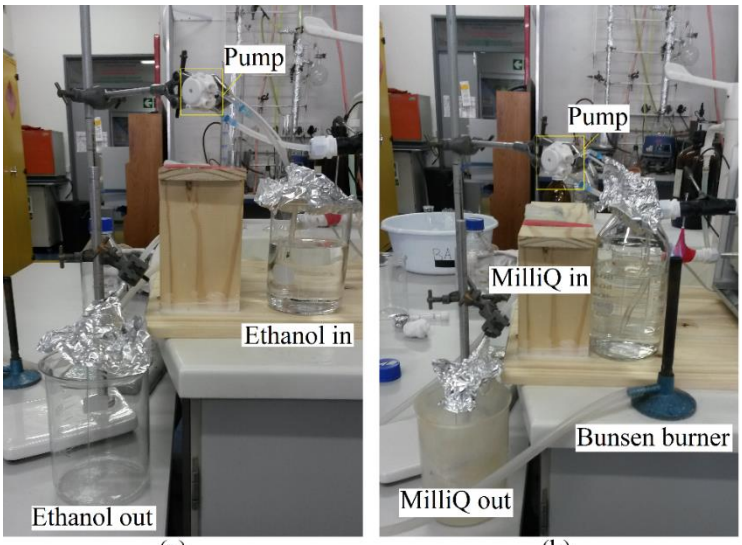


Figure 9.13: (a) Flushing the coil with 70% ethanol; (b) Flushing the coil with sterile water

The subculture is diluted and the inoculation volume is calculated as previously described in the preparations. Before the batches are inoculated, a 200 µl sample is taken from each batch as a positive control sample. A positive control shows the best case results, whereas a negative control would show the worst case for the tests.

Samples are taken by using a pipette set to the desired sample size. The sample is placed on the agar streak plate and spread evenly to cover the entire plate. The plate lid is then replaced and set aside.

The inoculation volume calculated previously is added to the batch using a pipette to inoculate the batch with the correct concentration of bacteria. A 200 µl sample of this inoculated media is then taken as a negative control.

The first batch is passed through the microwave setup at the set flow rate and power levels to achieve the desired exit temperature. The media is collected in a sterile bottle at the outlet and set aside. The second batch is then passed through the microwave setup and is also collected in a sterile bottle. Both batches are then left for a few minutes to cool down slightly so the agar would not melt when the samples are spread on the plates.

While the media cools down the coil is again sterilised by flushing it with 70% ethanol followed by sterile milliQ water and the ends are covered. A sample of each bottle is then taken and spread on an agar plate.

The first batch is passed through the microwave setup again and collected in a new sterile bottle and the same is done for the second batch. Again the coil is sterilised and the media batches left to cool before samples are taken.

All of the agar plates are placed in the incubation room, which is kept at 37 °C, for 24 hours to allow any remaining organisms to grow colonies. After 24 h passed the plates are inspected to determine if sterilisation was achieved. This can be done by counting the colonies on each plate and comparing the count to the number of colonies on the negative control. Photographs of each plate are taken with the setup used in Figure 9.14 to record the results.

Figure 9.14: Camera setup used to photograph the petri dishes after incubation

9.4.2 AlamarBlue Tests:

AlamarBlue is a liquid that can be used to determine the metabolism of organisms in a sample. The liquid is dark blue in inactive media, but will turn red when metabolised by organisms.

A 96-well plate is used for these tests, this plate has small wells in a 12x8 grid. Each well is filled with a 100 µl sample and 10 µl of AlamarBlue is used per sample. The layout of the well plate can be seen in Figure 9.15.

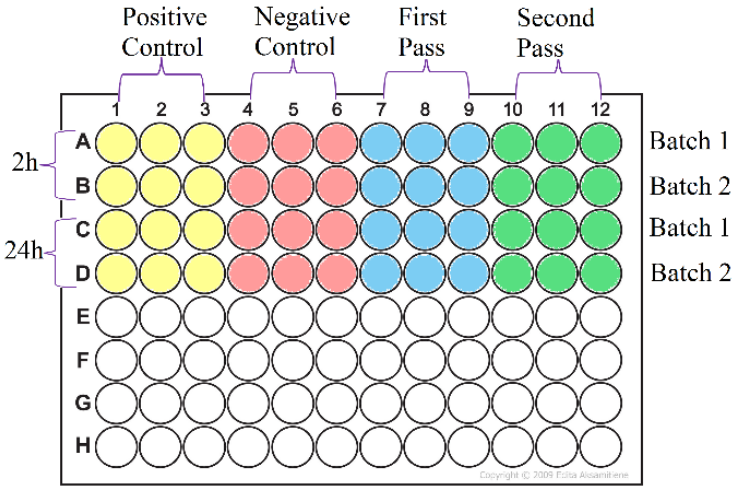


Figure 9.15: 96-well plate layout for AlamarBlue tests

These tests are done simultaneously with the streak plate tests. It was decided to fill three wells with each sample case: positive and negative controls as well as for each microwave pass of each batch. Two rows for each batch were done for different incubation times.

Once all the samples have been collected the plate is left in the incubation room at 37 °C for one hour. The plate is removed and the AlamarBlue added to the first two rows to be tested. The plate is then left for another hour to incubate further, after which it is placed in a fluorescent plate reader which generates the results. The fluorescent readings are low for inactive wells and high for active wells.

The plate is removed from the plate reader and left for further 21 h to incubate. After 21 h AlamarBlue is added to the last two rows and the plate is left to incubate for the final hour. This brings the incubation time of the plate up to a total of 24 h. Again, the plate is placed in the florescent plate reader to obtain the results.

Figure 9.16 shows a well plate after the tests for 24 h was completed. Note the red colour of the active negative control wells vs the dark blue of the inactive positive controls.

Figure 9.16: Well plate used in AlamarBlue tests

These tests were done for the higher concentrations of microorganisms as a possible additional confirmation that the organisms died. The tests may be able to provide faster results in comparison to the streak plates.

9.5 Test Summaries and Results

Following the above described test procedures, the following tests were conducted. First all three microorganisms were tested at 90 °C and a flow rate of 3.5 l/h. Tests at lower temperatures were conducted next using *M. Luteus*. Lastly, tests at 50 °C at lower and higher flow rates were conducted to test the effect of microwave exposure time. These tests are summarised in Table 9.1.

Table 9.1: Test summary

Test number	Target temperature	Flow rate	Microorganism used
1	90 °C	3.5 l/h	Unsterilised growth media
2	90 °C	3.5 l/h	*M. Luteus*; 10^3 cells per ml
3	90 °C	3.5 l/h	*M. Luteus*; 10^6 cells per ml
4	90 °C	3.5 l/h	*E. Coli*; 10^6 cells per ml
5	90 °C	3.5 l/h	*S. Cerevisiae;* 10^6 cells per ml
6	70 °C	3.5 l/h	*M. Luteus*; 10^6 cells per ml
	50 °C	3.5 l/h	*M. Luteus*; 10^6 cells per ml
	37 °C	3.5 l/h	*M. Luteus*; 10^6 cells per ml
7	50 °C	2 l/h	*M. Luteus*; 10^3 cells per ml
	50 °C	8 l/h	*M. Luteus*; 10^3 cells per ml

9.5.1 Test 1

Firstly, a batch of unsterilised TSB media was tested to confirm that the media is not affected negatively by the microwaves. This test was only a single batch of 250 ml and was passed through the microwave setup twice. The positive control for this test was a sample of the media that had been autoclaved. By visual inspection the media was unchanged in colour. Samples of each pass were taken for streak plates and the results can be seen in Figure 9.17.

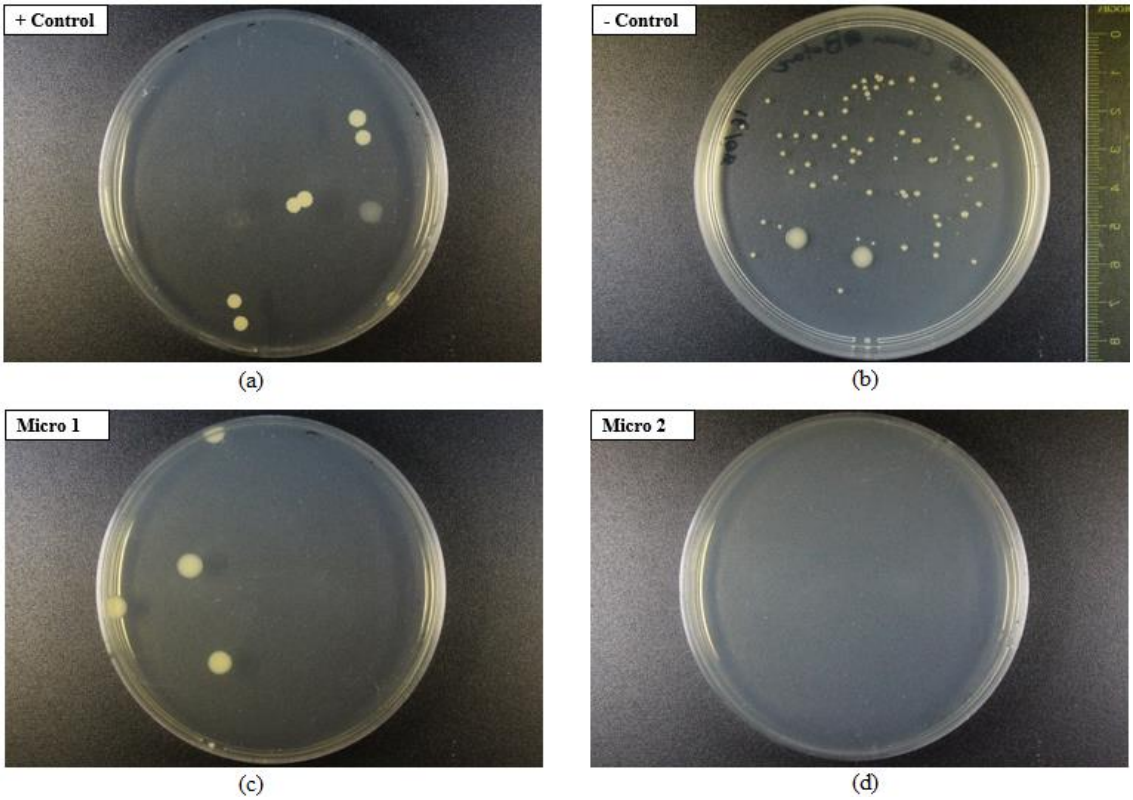


Figure 9.17: Results of media tests: (a) Positive control; (b) Negative control; (c) Microwave pass 1; (d) Microwave pass 2

From the streak plates it is confirmed that the microwave system kills microorganisms in the media. The colonies seen in the positive control were identified as fungi spores. These spores are not always killed by the autoclave. The microwave system killed all the microorganisms during the first pass except for these spores. However, after the second pass, the streak plate is completely clean, suggesting that the microwave system may be able to kill spores if the exposure time is longer.

9.5.2 Test 2

The second test was with *Micrococcus Luteus* using a 10^3 cells per ml concentration. This means there is 10^3 bacteria cells per millilitre of media. At an OD_{620nm} of 0.2, *M. Luteus* has a concentration of 1.3 x 10^7 cells per ml. By using this value, the inoculation volume needed to achieve the desired concentration in the 250 ml batch was found to be 19.23 µl.

The results of the streak plates for batch one is shown in Figure 9.18 and for batch two in Figure 9.19.

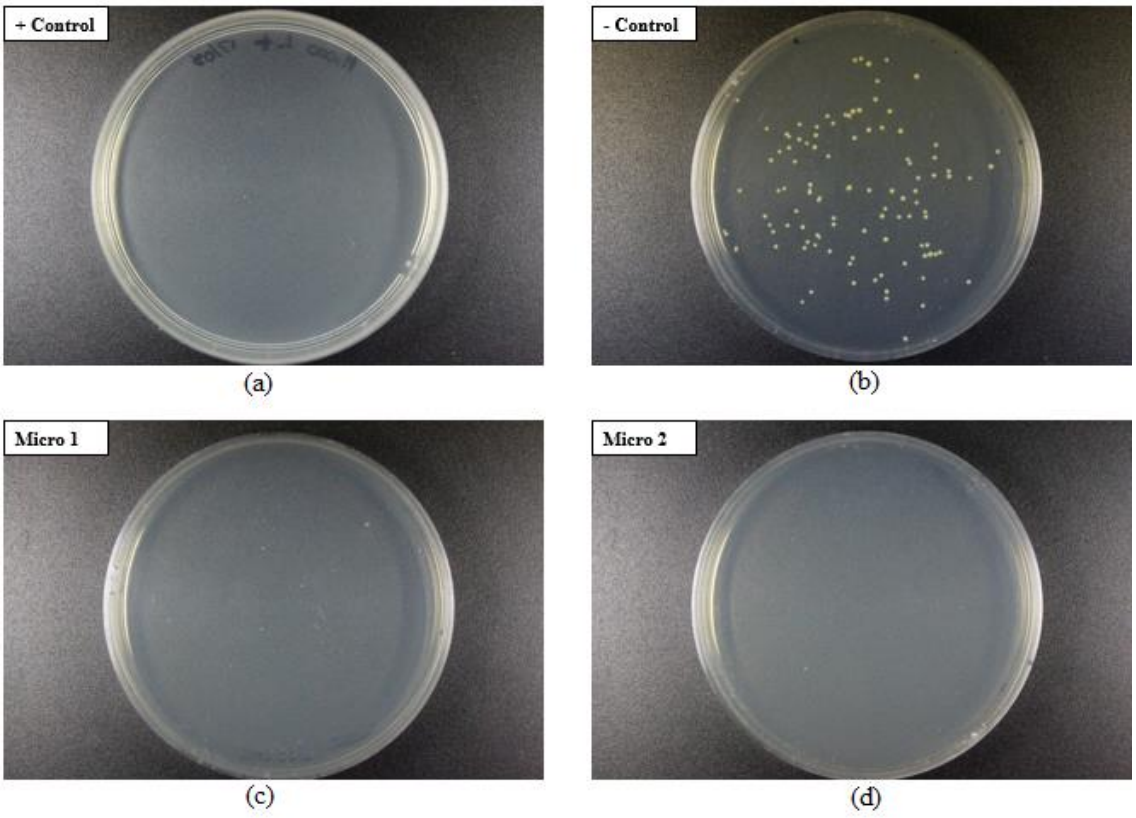


Figure 9.18: Results of *M. Luteus* 10^3 Batch 1: (a) Positive control; (b) Negative control; (c) Microwave pass 1; (d) Microwave pass 2

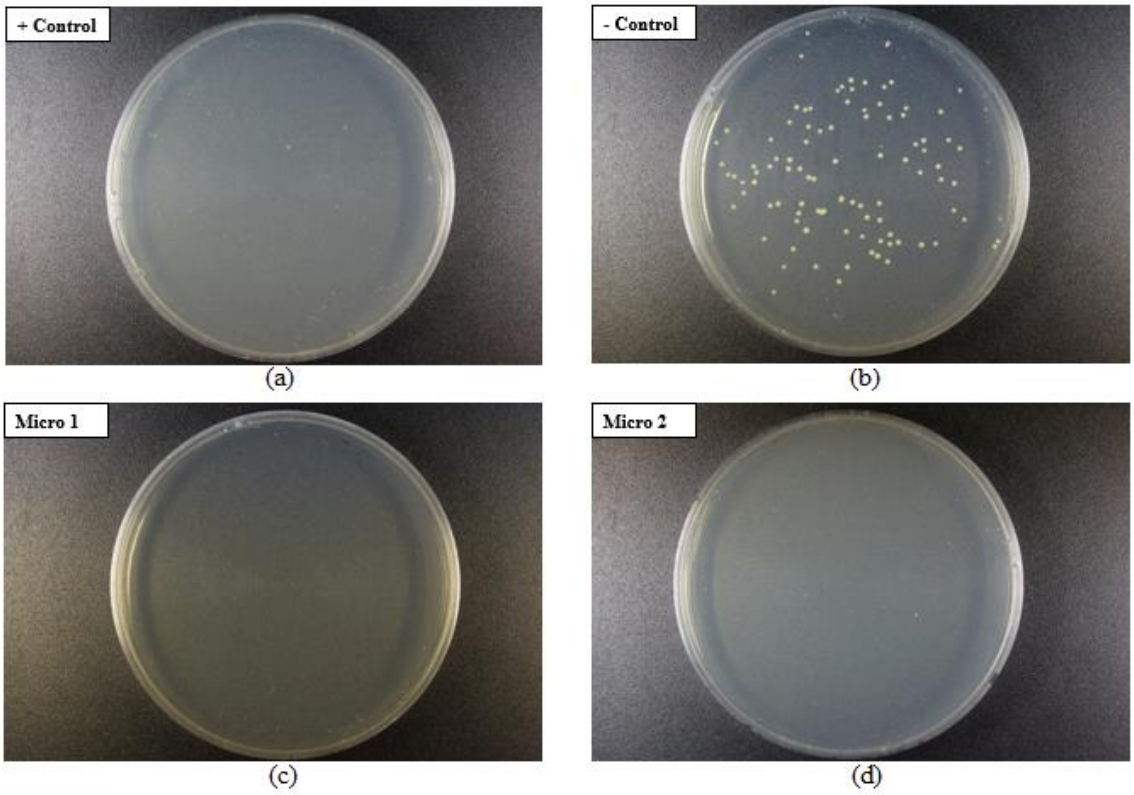


Figure 9.19: Results of *M. Luteus* 10^3 Batch 2: (a) Positive control; (b) Negative control; (c) Microwave pass 1; (d) Microwave pass 2

As seen in the results the microwave system kills all bacteria in the first pass. After these tests it was decided to increase the concentration to 10^6 cells per ml for the next test.

9.5.3 Test 3

Still using *M. Luteus* the new inoculation volume needed was calculated to be 19.23 ml per 250 ml batch.

Because this is such a high concentration it would be impossible to count the cells on a streak plate. Therefore, the samples of the negative control as well as the two microwave setup passes were diluted 1000 times. This was done in two stages: first by taking a 10 µl sample and adding it to 990 µl of sterile media which dilutes the sample 100 times. A 100 µl sample of this dilution is then added to 900 µl of sterile media for a further 10 times dilution. 200 µl of this final dilution is used on the streak plate. Figure 9.20 and Figure 9.21 shows the streak plates of batch one and batch two, respectively, with streak plates of the microwaved samples both undiluted and diluted.

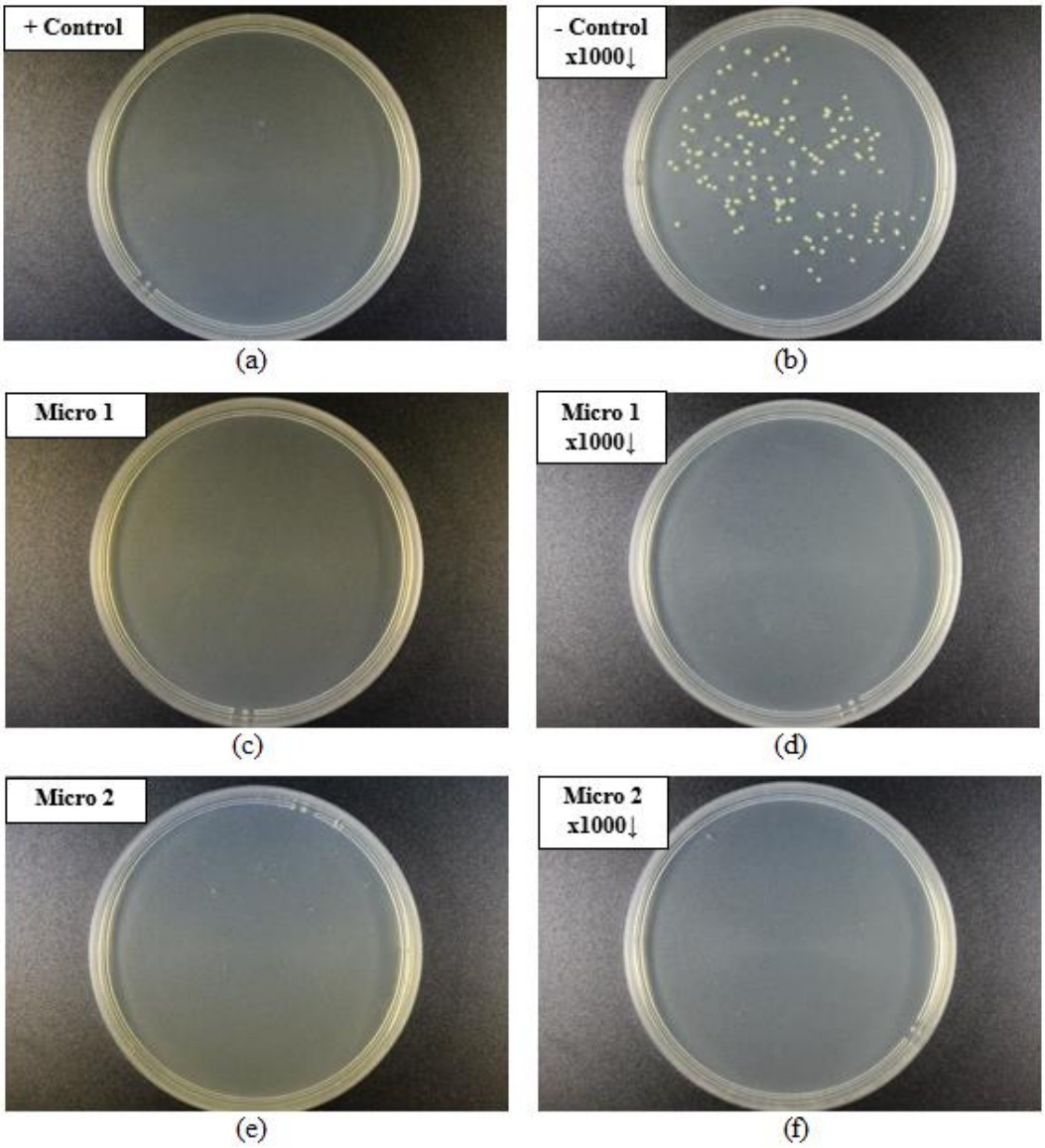


Figure 9.20: Results of *M. Luteus* 10^6 Batch 1: (a) Positive control; (b) Negative control, 1000 times diluted ; (c) Microwave pass 1, undiluted; (d) Microwave pass 1, 1000 times diluted (e) Microwave pass 2, undiluted; (f) Microwave pass 2, 1000 times diluted

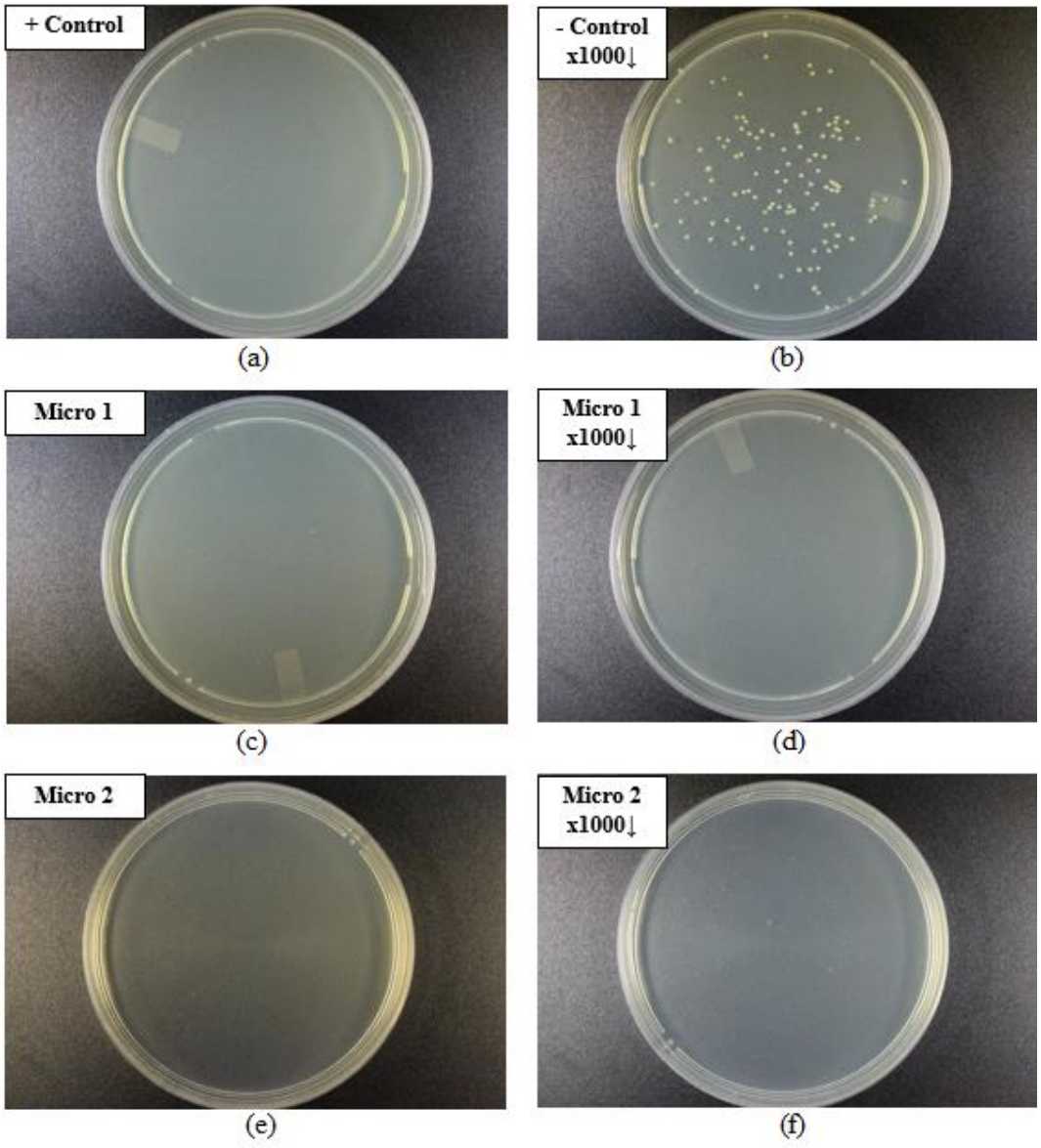


Figure 9.21: Results of *M. Luteus* 10^6 Batch 2: (a) Positive control; (b) Negative control, 1000 times diluted ; (c) Microwave pass 1, undiluted; (d) Microwave pass 1, 1000 times diluted (e) Microwave pass 2, undiluted; (f) Microwave pass 2, 1000 times diluted

From these results it is clear that the microwave sterilisation method is effective in sterilising gram positive bacteria. AlamarBlue tests were also done for this concentration. The wells were filled with undiluted samples as the cells will not be counted. Figure 9.22 shows the results of the 2 h and 24 h AlamarBlue tests.

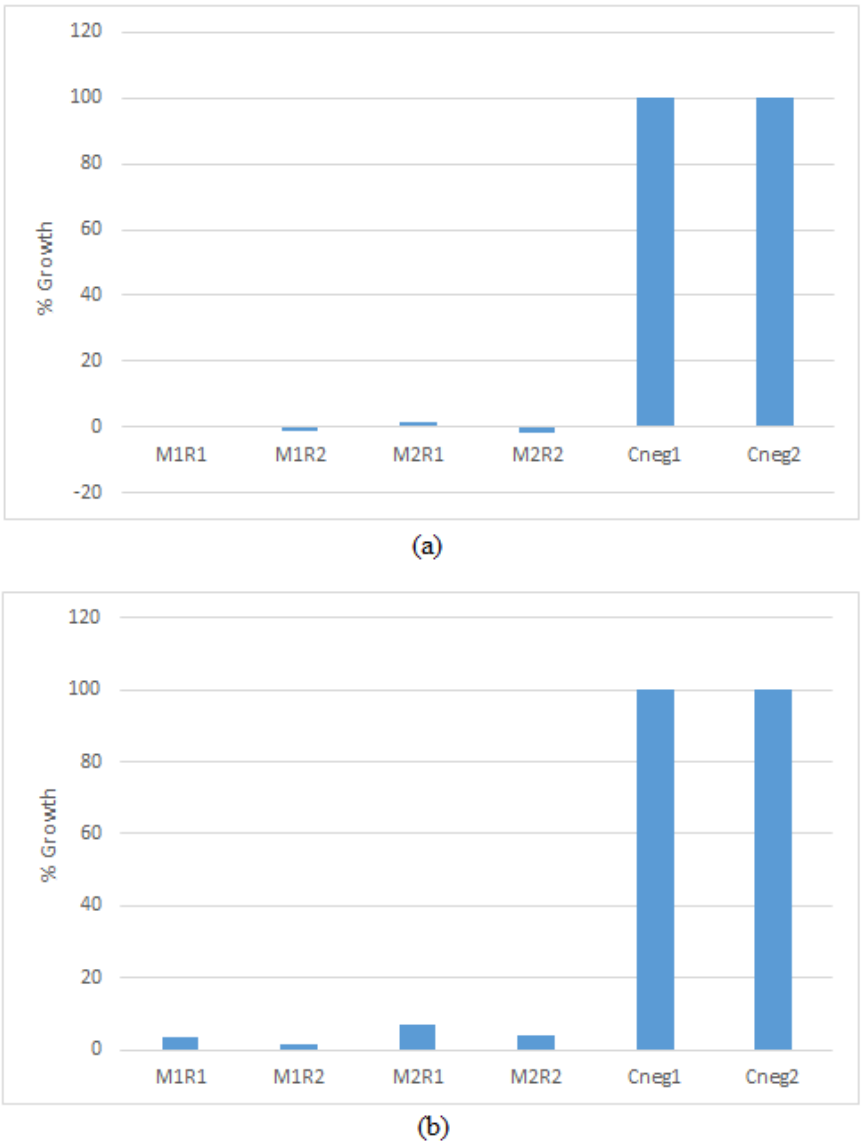


Figure 9.22: AlamarBlue results for *M. Luteus* 10^6: (a) For 2 h incubation time; (b) For 24 h incubation time.

The 2 h incubated tests yielded good results with growth only in the negative controls, Cneg1 and Cneg2. M1R1 and M1R2 are the first and second microwave passes of batch one. M2R1 and M2R2 is the first and second passes of batch 2, respectively.

In the 24 h incubated results some growth is observed in the test samples. Since the streak plates of the tests were still clean after 24 h the growth observed in this test is possibly due to contaminants falling into the wells while the plate was tested after 2 h.

9.5.4 Test 4

The next bacteria to be tested was *Escherichia Coli* at a 10^6 cells per ml concentration. *E. coli* has a concentration of 7.8 x 10^7 at an OD_{620nm} of 0.2. This results in a 3.2 ml inoculation volume added to the 250 ml batch. Again, samples for the streak plates were diluted 1000 times. Figure 9.23 and Figure 9.24 shows the plates for batch one and batch two respectively.

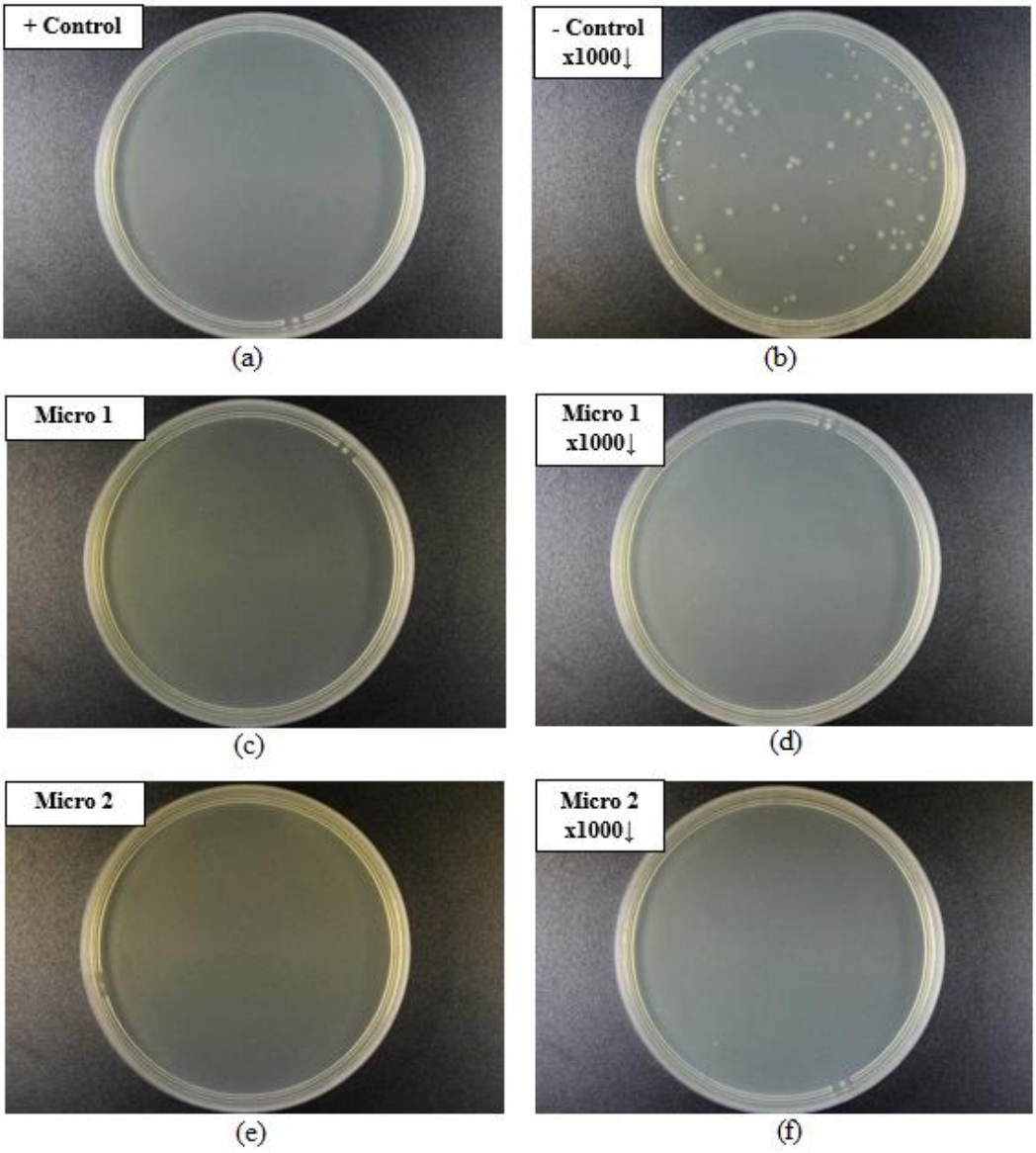


Figure 9.23: Results of *E. Coli* 10^6 Batch 1: (a) Positive control; (b) Negative control, 1000 times diluted ; (c) Microwave pass 1, undiluted; (d) Microwave pass 1, 1000 times diluted (e) Microwave pass 2, undiluted; (f) Microwave pass 2, 1000 times diluted

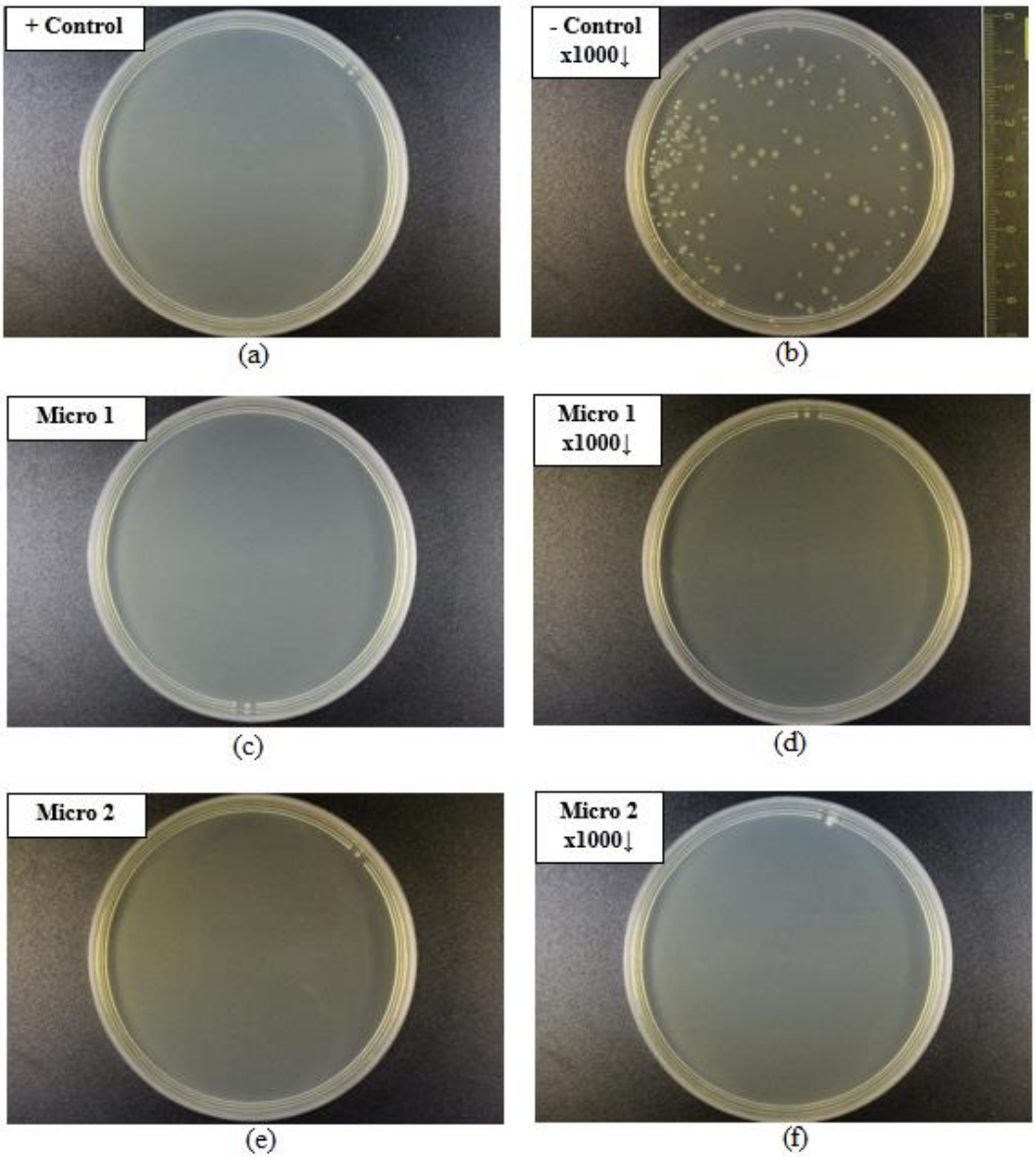


Figure 9.24: Results of *E. Coli* 10^6 Batch 2: (a) Positive control; (b) Negative control, 1000 times diluted ; (c) Microwave pass 1, undiluted; (d) Microwave pass 1, 1000 times diluted (e) Microwave pass 2, undiluted; (f) Microwave pass 2, 1000 times diluted

AlamarBlue tests were also done on this bacteria concentration at 2h and 24h incubation. Unfortunately the wells for the 2 h positive control seemed to be contaminated as they showed significant growth. The growth seen in the 2 h test is disproved by the clear streak plates after 24 h and by the AlamarBlue test after 24 h as seen in Figure 9.25.

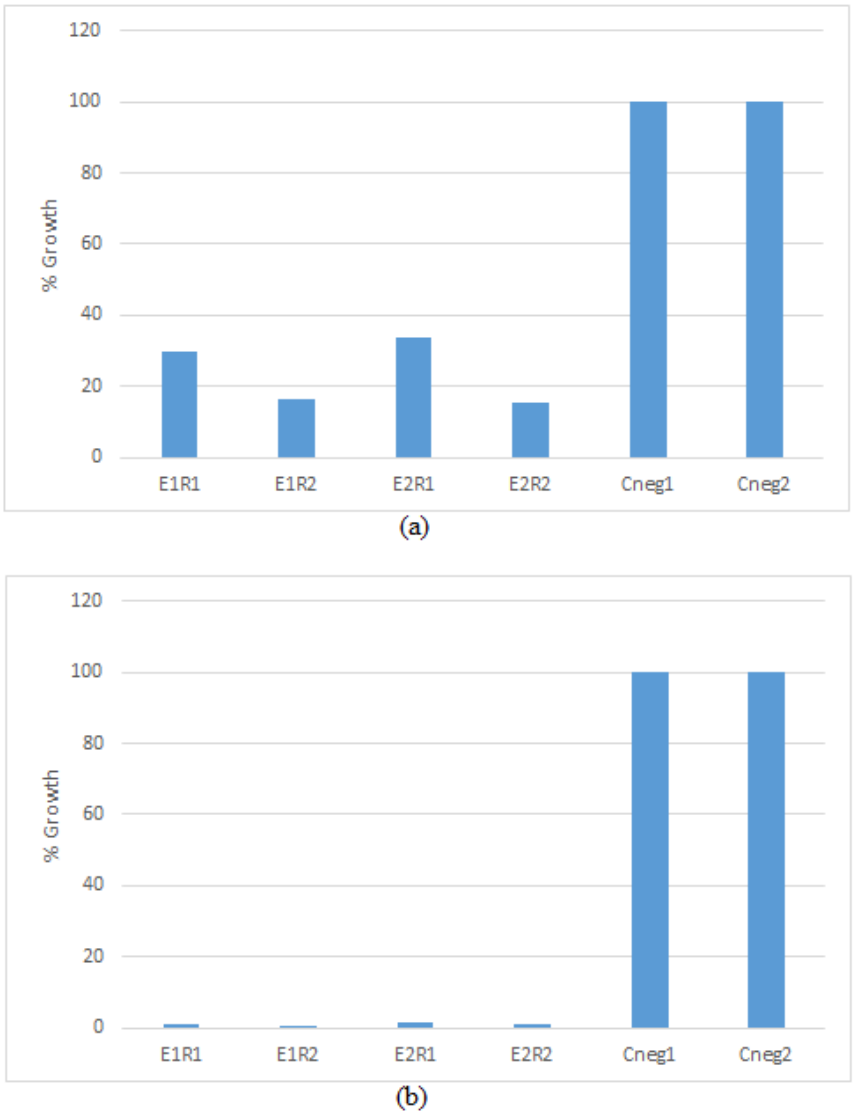


Figure 9.25: AlamarBlue results for *E. Coli* 10^6: (a) For 2 h incubation time; (b) For 24 h incubation time.

These tests prove that the microwave system can kill gram positive bacteria as well.

9.5.5 Test 5

The next test will be *Saccharomyces Cerevisiae* also at a concentration of 10^6 cells per ml. *S. Cerevisiae* at an OD_{620nm} of 0.2 has a concentration of 3 x 10^8 leading to a required inoculation volume of 0.83 ml per 250 ml batch. The yeast has a very long generation time, which means the streak plates had to be incubated for 48 h to grow visible colonies. The results can be seen in Figure 9.26 for batch one and Figure 9.27 for batch two.

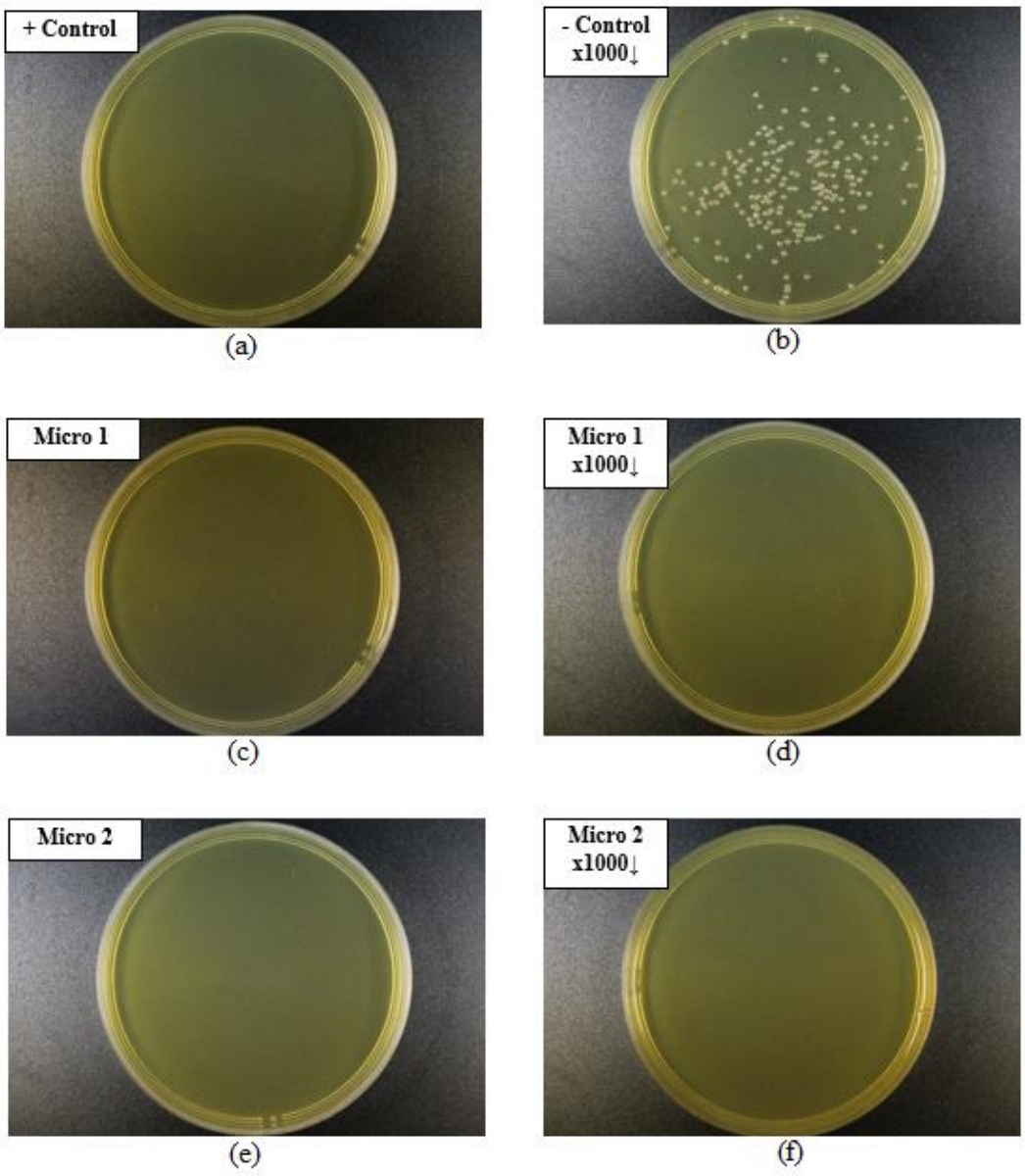


Figure 9.26: Results of *S. Cerevisiae* 10^6 Batch 1: (a) Positive control; (b) Negative control, 1000 times diluted ; (c) Microwave pass 1, undiluted; (d) Microwave pass 1, 1000 times diluted (e) Microwave pass 2, undiluted; (f) Microwave pass 2, 1000 times diluted

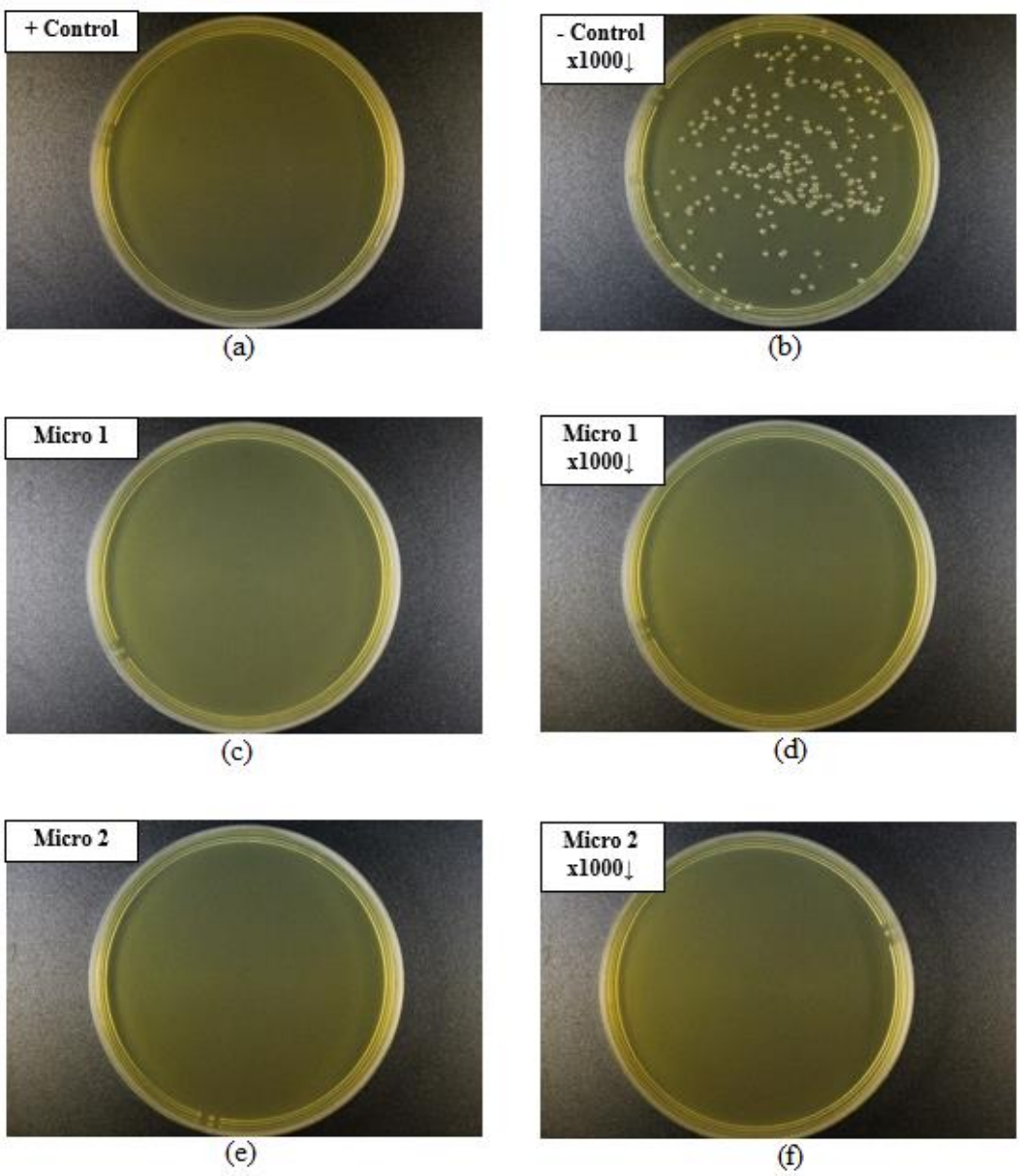


Figure 9.27: Results of *S. Cerevisiae* 10^6 Batch 2: (a) Positive control; (b) Negative control, 1000 times diluted ; (c) Microwave pass 1, undiluted; (d) Microwave pass 1, 1000 times diluted (e) Microwave pass 2, undiluted; (f) Microwave pass 2, 1000 times diluted

These plates show that the microwave setup kills yeast as well. This was supported by the AlamarBlue test after 24 h incubation as seen in Figure 9.28. Since the yeast grows so slow, the 2 h test was omitted.

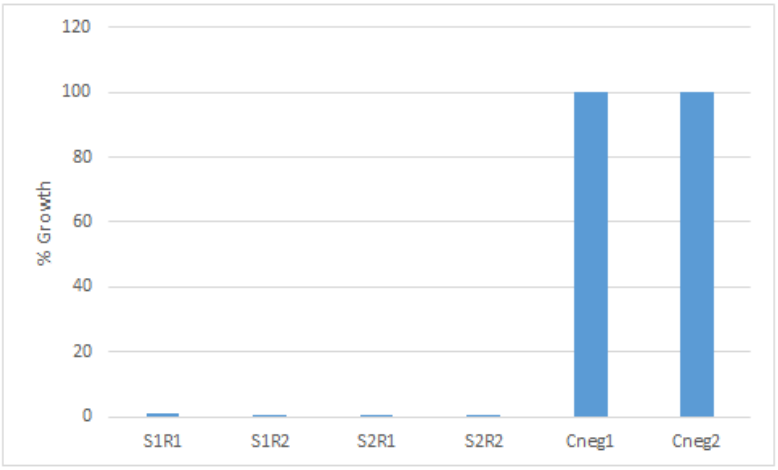


Figure 9.28: AlamarBlue results for *S. Cerevisiae* 10^6 for 24 h incubation time

9.5.6 Test 6

Since all three types of microorganism were successfully killed at 90 °C it was decided to do test at lower temperatures. *M. Luteus* was selected to do these tests with since it is the simplest to grow in the lab. Three temperatures over a wide range were selected to determine at which temperature the microwave setup no longer kills bacteria. This range is: 70 °C, 50 °C and 37 °C. These temperatures were still tested in duplicate, but to simplify the testing procedure each batch was only passed through the microwave setup once. Additionally, only the negative controls were diluted. Figure 9.29 shows the results for 37 °C, Figure 9.30 shows the results for 50 °C and Figure 9.31 shows results of 70 °C.

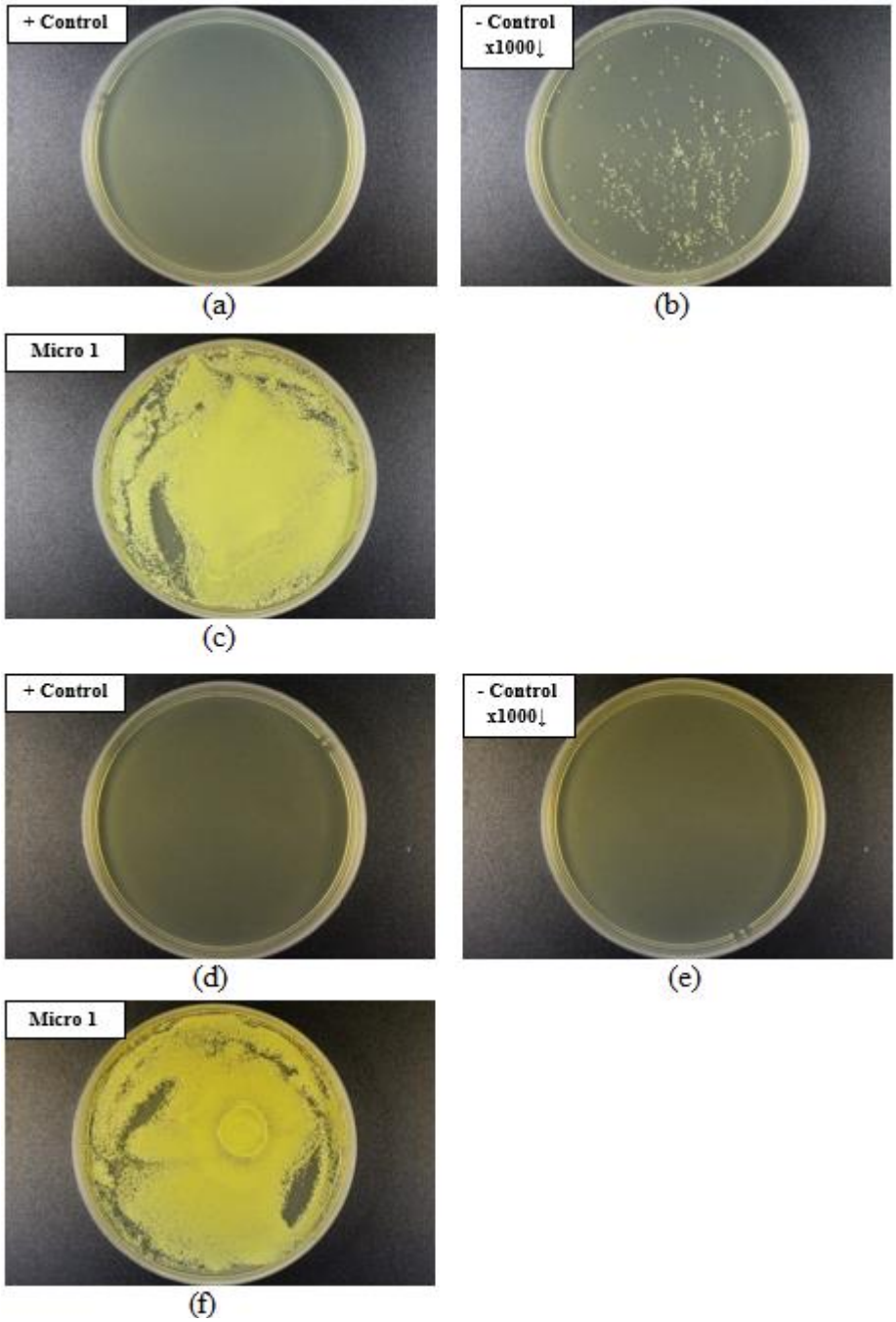


Figure 9.29: *M. Luteus* 10^6 at 37 °C: Batch 1: (a) Positive control; (b) Negative control, 1000 times diluted; (c) Microwave pass 1, undiluted; Batch 2: (d) Positive control; (e) Negative control, 1000 times diluted; (f) Microwave pass 1, undiluted

Note: Batch two's negative control sample was overlooked when inoculation was being done on the samples, thus nothing is growing on the negative control. This control should represent the same amount of cells as the negative control of batch one.

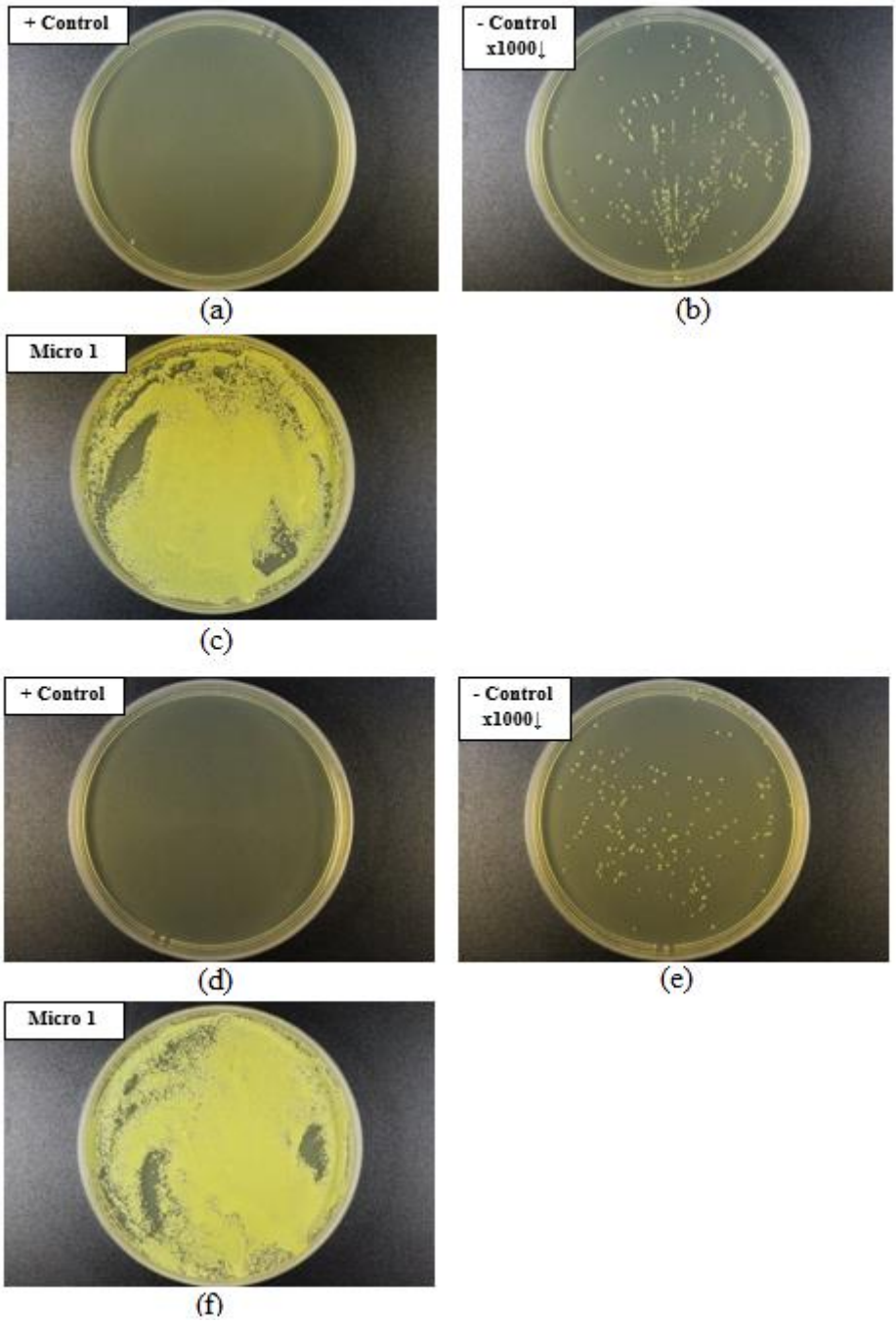


Figure 9.30: *M. Luteus* 10^6 at 50 °C; Batch 1: (a) Positive control; (b) Negative control, 1000 times diluted; (c) Microwave pass 1, undiluted; Batch 2: (d) Positive control; (e) Negative control, 1000 times diluted; (f) Microwave pass 1, undiluted

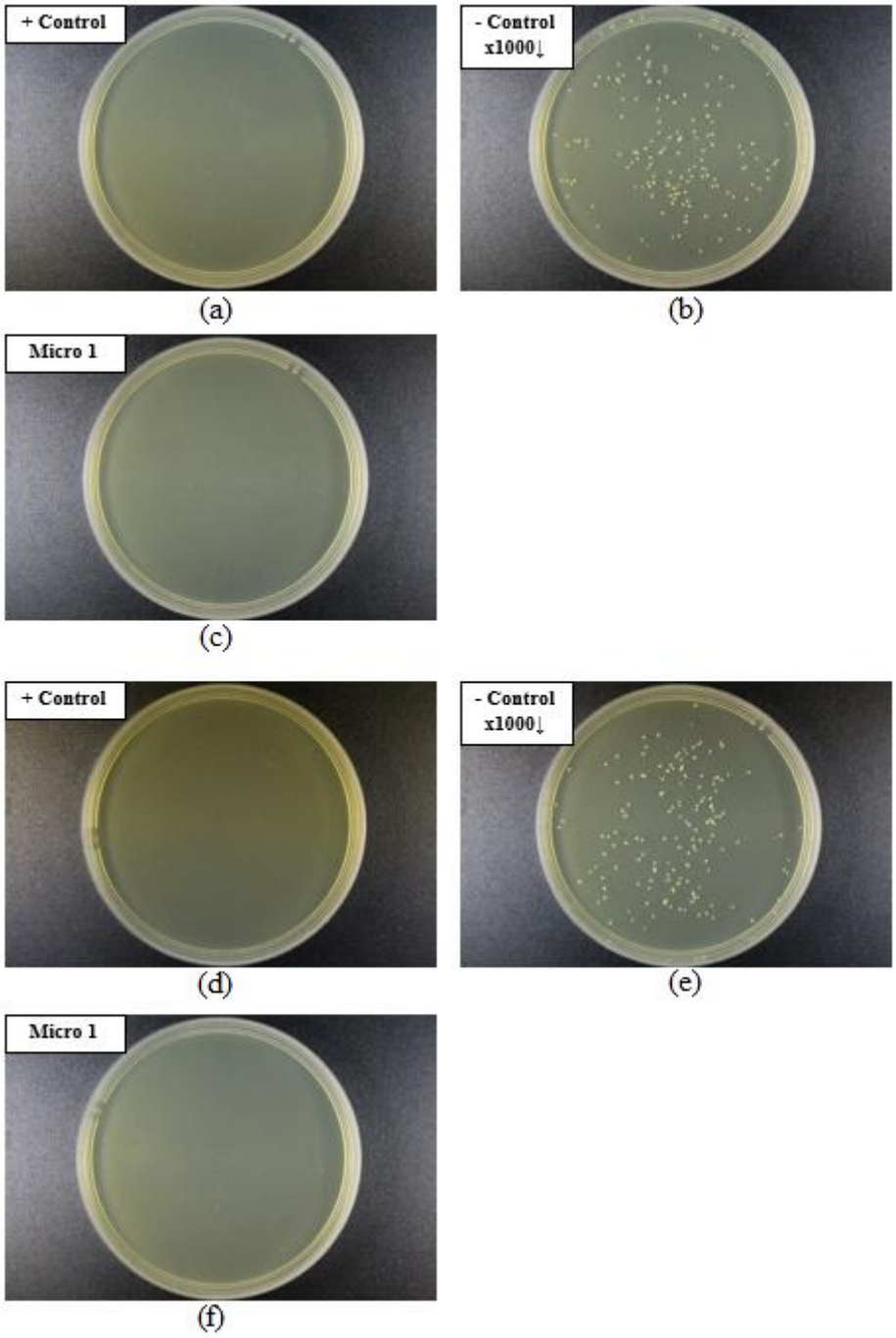


Figure 9.31: *M. Luteus* 10^6 at 70 °C; Batch 1: (a) Positive control; (b) Negative control, 1000 times diluted; (c) Microwave pass 1, undiluted; Batch 2: (d) Positive control; (e) Negative control, 1000 times diluted; (f) Microwave pass 1, undiluted

As can be seen from these results, it seems that microwave sterilisation still requires temperatures above 70 °C to assist in killing microorganisms.

9.5.7 Test 7

Two cases were tested:

The first was to test was done to determine if longer exposure to microwaves has any effect on the microorganisms below 70 °C. This was done by setting the target exit temperature at 50 °C and selecting a lower flow rate of 2 l/h. This means the media is exposed to lower power but for a longer time.

The second cases tested if higher microwave power levels have any effect on the microorganisms below 70 °C. This was achieved by also setting the target exit temperature to 50 °C but using a higher flow rate of 8 l/h. This will reduce the exposure time, but increases the power to which the media is exposed.

Both cases were tested using *M. Luteus* at a 10^3 cells per ml concentration which means no dilution is necessary. Each batch was passed through the microwave setup twice and the results are displayed in Figure 9.32.

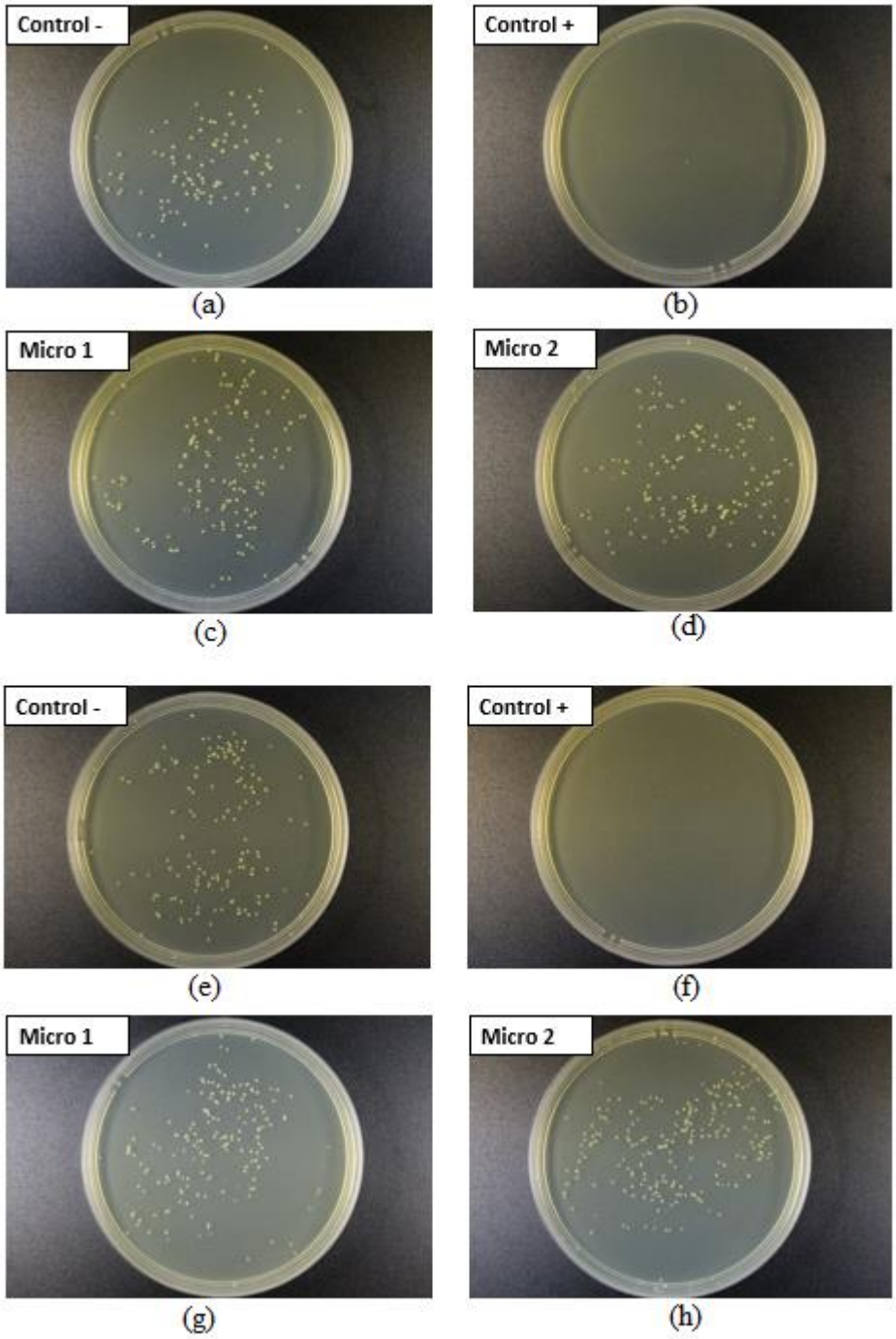


Figure 9.32: *M. Luteus* 10^3 at 50 °C- Low flow rate, Low power: (a) Negative control; (b) Positive control; (c) Microwave pass 1 (d) Microwave pass 2; High flow rate, High Power: (e) Negative control; (f) Positive control; (g) Microwave pass 1; (h) Microwave pass 2

As seen in the streak plates neither of the changes effected the bacteria, again pointing to the fact that heat is required to kill them.

9.6 Energy Efficiency

The energy use of the microwave setup and the autoclave was calculated for a batch of 10 litres to compare their energy efficiency. The energy of the microwave setup was calculated for different flow rates and different power levels. First the transformer input power was calculated for each magnetron output power level. The different input powers were calculated using the microwave setup's efficiency of 58.5%.

$$P_{in} = \frac{P_{out}}{0.585} \quad \textbf{9-2}$$

The time it takes to process the batch, t_{batch}, is determined by:

$$t_{batch} = \frac{V_{batch}}{\dot{V}} \quad \textbf{9-3}$$

Where V_{batch} is the batch volume and $\dot{V}$ is the flow rate.

Finally, the energy used can be calculated as:

$$E = P_{in} t_{batch} \quad \textbf{9-4}$$

Figure 9.33 shows the energy used for different power levels and different flow rates.

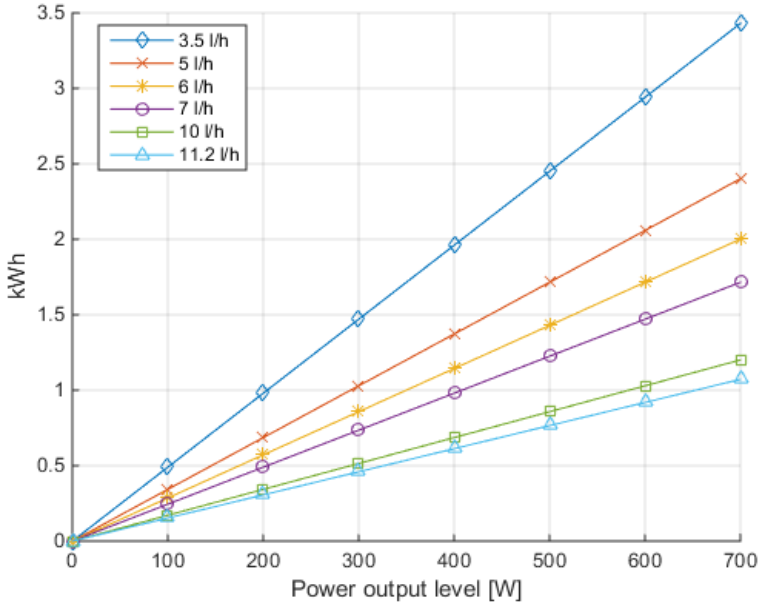


Figure 9.33: Energy required to process 10 *l* at different flow rates

The autoclave used in the Department is rated 21 kW. This autoclave has a sterilisation time of 30 min when used for liquids. This does not include the heating and depressurising times, which brings the total operating time up to 60 min. This autoclave can sterilise 10 litres of media in a single use. Using these values in eq. 9-4 the autoclave uses 21 kWh.

From the heating predictions in Chapter 6: Heat Transfer, Figure 6.2 a flow rate of 10 l/h will reach 70 °C at 650 W. Based on this and the results of the sterilisation tests that 70 °C is sufficient for sterilisation, the microwave setup uses 1.1 kWh to sterilise the same batch size in the same time.

9.7 Chapter Closing

This chapter gave detailed descriptions of the test preparations and test procedures before discussing the individual tests and their results. The microwave setup was compared to the autoclave used the Department in terms of energy use.

The sterilisation test results show that the developed microwave method is capable of killing both gram positive and gram negative bacteria as well as yeast. The possibility exists that it could also kill fungi spores, as seen in the very first test. Fungi was not tested in this setup as it would be very difficult to ensure no spores are released into the lab while working at the microwave setup. It would also require different methods of sterilising the coil after each batch as ethanol does not kill spores.

This method of sterilisation is faster and more instant than autoclaving. Working at 90 °C and 3.5 l/h, a batch of 250 ml media can be sterilised in 4.2 min. There is also less potential danger as there is no pressure in the system. Due to this short heating time, none of the media was negatively affected by the microwaves in terms of caramelisation and the Maillard reaction which would cause discolouration.

It was also shown that the microwave system can be more energy efficient than an autoclave and if faster flow rates are used, the system could match the volume sterilised by the autoclave within the same time.

Chapter 10:

Recommendations and Conclusion

10.1 Chapter Summary

This chapter will discuss recommendations for future developments in this field. The project is concluded by confirming that the objectives have been reached, discussing possible alternative applications for the developed concept and summarising the final results.

10.2 Recommendations

The following recommendations can be used to improve the system in future projects and for potential commercialisation.

10.2.1 User Interface

An easy to use interface should be programmed, which allows the user to easily change any settings. This should preferably be done using open source software as opposed to a licenced program that not everyone can use.

10.2.2 Variable Volume

The system should be adaptable to use different coil sizes. The system should be designed in such a manner that the coils can be easily changed by the user without errors that may cause hazardous microwave leakage.

10.2.3 Relate Voltage to Power

In Chapter 3: Measurement Setup and Microwave Power Control, the relationship between the anode voltage and the magnetron output power was briefly investigated. Further studies into this relationship may yield new methods of measuring power without detector diodes.

10.2.4 Mode Stirrer Design

If the system is scaled up for industrial process lines, proper investigation of the effect of mode stirrer design should be done. Simulated models of different mode stirrer designs may be beneficial for large scale system designs with different microwave cavities.

10.2.5 Replacement Pump

A peristaltic pump with accurate flow rate control should be designed or sourced if the system is to be commercialised. The pump should be able to send the current flow rate back to the controller and automatically adjust the flow rate if needed.

10.2.6 Automate Microwave Oven Switch On

In the current microwave system, the user has to manually switch on the microwave oven. For large scale implementation this should be automated by the controller. Sensors should be placed either inside the coil or at the inlet to confirm that there is fluid inside the cavity. This will prevent the microwave from being switched on with an empty cavity which may damage the magnetron.

10.2.7 Non-thermal Microwave Effects

The non-thermal effect of microwaves can be investigated using a system that has identical heat transfer rates, flow rates and volumes as the microwave system. Therefore the only difference between the systems is the method of heating.

10.2.8 Alternative Approach to Control Temperature

In this system design the exit temperature was controlled by changing the microwave power into the cavity while keeping the flowrate constant. It should also be possible to control the temperature through keeping a constant microwave power level and changing the flow rate. This could possibly result in similarly effective system but with less control circuitry.

It should be noted that if the application is for a continuous flow process as part of a production line, it may not be possible to change the flow rate without the use of storage containers since the entire line's flowrate may not be able to change. s

10.2.9 Sterilisation Tests

For future sterilisation tests a different setup should be used that can be operated inside a laminar flow cabinet to safely work with fungi spores. This system should also allow the coil to be removed as this eases the sterilisation process of any remaining spores in the coil.

More extensive tests should be done on the media to document the effect of microwave heating on the nutrient levels. These tests could include UV spectrometry to show discolouration and amino acid and glucose analysis to quantify the loss of nutrients.

10.3 Conclusions

10.3.1 Objectives

The stated objectives for this project were met. A continuous flow sterilisation system has been developed which uses only microwaves as a source of heat. The final system was assembled using a modified domestic microwave oven with a coiled pipe to allow the media to be pumped through the cavity. The microwave output power was controlled by the developed anode current control method. Finally, sterilisation tests were done on contaminated growth media, which proved that the system is successful as a method of sterilisation.

10.3.2 Future Applications

This proof of concept design can be further developed into a commercial product for either large scale continuous process lines or as a small lab appliance that can quickly sterilise any size batch of growth media.

The concept of sterilisation can also be applied to other fields such as the food industry in order to sterilise milk of fruit juices. This can also be applied to manufacturing lines where water is used to cool down equipment such as saws or drills. In these situations the unsterilised water can form biofilms within the water pipes which cause blockages and results in the equipment not being cooled properly. If the water is sterilised before it enters the pipes the biofilms will not form and the equipment will last longer [19].

10.3.3 Final Conclusion

The developed microwave system was successfully used in sterilisation tests conducted on different types of microorganisms at high concentrations. All tests that were done above 70 °C completely sterilised the media without causing any visible damage to it.

It was also shown that even though microwave ovens themselves are not very efficient in terms of power, with a 58.5% efficiency, microwaves as a method of sterilisation is more energy efficient than the autoclave currently used in the Department.

Industry accepts a Sterility Assurance Level (SAL) of 10^{-3} as sterile [19]. This developed microwave sterilisation method achieved a SAL of 10^{-6} at temperatures above 70 °C. At the end of the project, it can be seen that the proposed method of sterilising media by using only microwaves was successful.

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Appendix A:

Safety

A.1 Section summary

Before any work is started a safety analysis should to be done on the project. This section will discuss the risks that were identified for this project. Safety precautions for each risk is discussed and should be applied in any future projects in this field.

A.2 Risk analysis

A.2.1 RF Radiation

Leaking microwaves can cause skin burns, deep tissue burns and even blindness when leakage levels exceed the acceptable limits. The generally accepted safety limit for domestic microwave ovens is 5 mW/cm^2 at a distance 5 cm away [1]. All connections between parts such as an isolator and a dual-directional coupler should be insulated with aluminium tape to reduce the risk of leaking. A RF field monitor should be used to confirm that the work environment is safe. In this project a Field Sense personal RF monitor was used each time the microwave setup was switched on. This device has an alarm mode that will sound when the leakage is above acceptable limits.

The door of a microwave oven usually has a metallic grid of small holes. These holes are smaller than the microwave wavelength which means the microwaves cannot pass through them. The door grid is known as $\lambda/4$ (quarter wavelength) radiation trap and is designed to contain the microwaves within the cavity but still allow the user to see inside [1]. These types of radiation traps should be designed if the user wishes to see inside a setup safely.

Warning signs for RF radiation should be displayed in the work area and emergency stops should be wired into the setup.

A.2.2 Electricity

The 220 VAC_{rms} main lines are used in power control circuits and can cause severe, even lethal, electric shocks. The transformers used are step-up transformers that have an output of 3-4 kV. The high voltage part of the circuit should be isolated from the rest of the circuits. All high voltage wires have to be insulated at the connection points to ensure no open live wires are present. High voltage warning signs should be placed in the work area. When working on the setup, the entire system must be switched off and the emergency stop must be engaged as an extra precaution. Specialised probes must be used when measuring the high voltage components. The one used in this project is a Fluke 6000V high voltage probe.

A.2.3 Lone working

If the operator is working alone in the lab, reasonable care must be taken to look after their own health and safety. Tools and other equipment must be used properly and in accordance with any relevant safety instructions.

A.2.4 High temperatures

If liquids are being heated in a system, the temperatures of the liquids or steam released can cause serious burn wounds. If a steam valve is present in the setup, it should be faced away from the operator's work area. Warnings should be placed in the work area and the liquid container should be placed in a safe area where accidental contact will be minimised.

A.2.5 Environmental

Any biological or chemical materials and liquids that will be used during the project development cannot be discarded untreated in a normal drain. All waste material and liquids should be labelled and set aside in a safe area during testing. These should be discarded according to the correct prescribed method which would differ for each material or liquid.